

SEDIMENT INORGANIC NITROGEN STOCKS AND ROOT-RHIZOME
AMMONIUM UPTAKE BY EELGRASS (*Zostera marina* L.)
IN THE LOWER CHESAPEAKE BAY

A Thesis

Presented to

The Faculty of the School of Marine Science
The College of William and Mary in Virginia

In Partial Fulfillment

Of The Requirements for the Degree of
Master of Arts

by

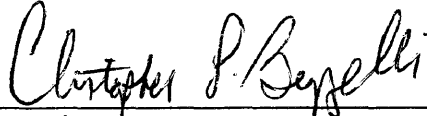
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1991

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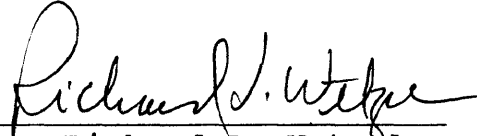
This thesis is submitted in partial fulfillment of
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Approved, October 1991



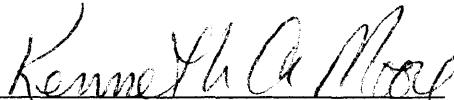
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ACKNOWLEDGMENTS

I gratefully acknowledge the guidance and support of my committee chairman and academic advisor, Dr. Richard L. Wetzel. Dick Wetzel provided the funding and laboratory space necessary to complete this project. Above these material allowances, Dick supplied the patience, leadership, and knowledge I required to gain practical experience and progress from a recent undergraduate to a developing marine ecologist. Thanks for everything Dick! I would also like to thank Ken Moore for his help in getting me acquainted with the greenhouse and for his overall knowledge of seagrass research. Howard Kator supplied the instruction necessary in using and maintaining the sometimes finicky ^{15}N vacuum manifold and in interpreting output from the JASCO emission spectrometer. Ken Webb's extensive experience in marine ecology and editorial techniques were of great benefit to me throughout this project. Pete Van Veld provided a large amount of moral support by continually complementing my progress. Drs. Hilary Neckles, Judson Kenworthy, and Frederick Short provided helpful ecological hints at various times during this study.

I am indebted to many VIMS personnel without whom I could never have completed this project. Foremost I would like to express my gratitude to Anna K. Kenne for immeasurable quantities of moral support and for her knowledge of digital cartography. Without her providing confidence and reminding me to keep an open mind, I would

not have matured as a scientist and a person. Thanks Anna! Lori Jedlica-Morris and Betty E. Berry provided much needed instruction in proper laboratory techniques and mental attitude. Art Schwarzschild, Jill Goodman, Chris Rowe, Cindi Horton, and John Field assisted in the field sampling, greenhouse set-up, and laboratory work and provided the camaraderie essential to the graduate student experience. Dr. Larry Haas and Dana Booth supplied expertise and patience in teaching me to run the CHN analyzer. The vessel operations staff were always amiable and kept the boats running smoothly. Sharon Dewing and Martha Craig provided friendship and "food for thought" throughout this study. Berch Smithson assisted with the digital map making and was always in good humor. A special acknowledgement goes to the College of Charleston in Charleston, S.C. for helping me become an organized and diligent worker. Finally, I would like to thank my parents, Guy and Mary Lou, for always encouraging me to do what made me happiest. Over the last three years, the actions and words of all the above people have contributed to my development as a person and a coastal aquatic ecologist.

Funding for this project was provided by the NOAA Office of Sea Grant, U.S. Department of Commerce, Grant No. NA90AA-D-SG045 to the Virginia Graduate Marine Science Consortium and Virginia Sea Grant College Program and from the general funding of the Virginia Institute of Marine Science by the Commonwealth of Virginia.

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I. ABSTRACT

Sediment NH_4^+ availability is one of several environmental factors that influence the growth and distribution of the seagrass, *Zostera marina* L. (eelgrass), in temperate marine and estuarine ecosystems. The objectives of this study were to inventory monthly sediment organic matter and inorganic nitrogen; quantify plant biomass and C:N ratios; and seasonally measure root-rhizome NH_4^+ uptake over a range of concentrations and incubation times.

From June 1990-May 1991 sediment organic matter and NH_4^+ (0-10 cm) ranged 0.70-1.64% and 51.20-349.1 μM , respectively. NO_x^- ranged 15.4-91.0 μM while plant biomass ranged 141.1-554.1 $\text{gdw} \times \text{m}^{-2}$. Shoot molar C:N ratio (12.3-21.1) and root-rhizome C:N ratio (15.6-27.6) also displayed monthly and seasonal variability. Plant biomass was significantly correlated to shoot C:N, sediment organic matter, and sediment NH_4^+ concentration.

Average root-rhizome NH_4^+ uptake rates were greatest during the autumn experiment (2.75 $\mu\text{mole N} \times \text{gdw RR}^{-1} \times \text{hr}^{-1}$) but winter uptake rates were greater than those of the spring experiment (1.88 vs 1.76). In all three experiments 4 hr uptake rates were higher than the 10 hr rates and uptake rate was a function of NH_4^+ concentration. All three experiments displayed Michaelis-Menten kinetics but the autumn V_{max} and K_s values (12.5 $\mu\text{mole N} \times \text{gdw RR}^{-1} \times \text{hr}^{-1}$ and 462 μM) were considerably higher than either the winter (3.29 and 23.4 μM) or the spring (3.3 and 44.8 μM).

The data suggest bi-directional influence between eelgrass and sediment NH_4^+ concentrations. Plant biomass contributes to the accumulation of sediment organic matter and enhances sediment NH_4^+ pools. An eelgrass seasonal nitrogen budget was developed using data from the literature and this study. In the fall, NH_4^+ uptake is greatest due to high sediment availability and reduced plant requirement. Eelgrass can absorb and store nitrogen through the root-rhizomes during the fall and winter for acripetal translocation in the spring. Although sediment NH_4^+ is usually in excess of plant demand, several other aspects of sediment chemistry (sulfide, phosphorus, rhizosphere nitrogen cycling) and plant physiology (above vs below ground production, internal translocation) may inhibit plant uptake potential.

II. PROJECT DESCRIPTION

A. INTRODUCTION

Seagrass meadows are prominent coastal aquatic communities, with annual primary production comparable to the world's most productive ecosystems. Seagrasses, phytoplankton, algal epiphytes, benthic microalgae, and algal macrophytes all contribute to net aquatic primary production in coastal areas, particularly in many estuaries. Seagrass biomass dampens turbulent flows and retains both autochthonous and allochthonous organic materials (Fonseca et al. 1983). Dense sediment microbial and meiofaunal assemblages regenerate essential inorganic nutrients from organic detritus. This community framework is ideal for the survival of ecologically and commercially valuable juvenile and adult fishes, invertebrates, and waterfowl as both refuge and feeding areas (Thayer et al. 1984; Heck and Thoman 1984; Orth and van Montfrans 1987).

Seagrass meadows integrate many physical, chemical, geological, and biological processes making their abundance and distribution direct indicators of ecosystem status. Anthropogenic coastal development and nutrient inputs generally degrade the clarity and overall quality of our coastal waters. Reduced light availability due to increased turbidity is a primary factor contributing to the decline in seagrass abundance and distribution in the Chesapeake Bay (Orth and Moore 1984). Despite inorganic nitrogen loadings

to estuaries, temperate estuarine systems are commonly described as having nitrogen limited net primary production. This is because nitrogen exists in several chemical pools due to biogeochemical processing (Howarth 1988). Parts of the Chesapeake Bay have been shown to demonstrate nitrogen limited production, but the possibility for spatial or temporal phosphorus limitation also exists (Howarth 1988). The dynamic nature of nitrogen supply and demand is particularly evident in vegetated areas as plant physiological processes influence sediment chemical cycles. Nitrogen is dynamically maintained in many organic and inorganic forms and is not always available for plant use.

B. BACKGROUND

Eelgrass (*Zostera marina* L.) is the dominant submersed vascular plant of the lower Chesapeake Bay (Orth and Moore 1984). Eelgrass production is primarily limited by light availability (Dennison and Alberte 1982), but irradiance interacts with temperature, rainfall, current patterns, and nutrient regimes to control plant growth. Whole plant biomass ranges from 60-550 gram dry weight x meter⁻² (gdw x m⁻²) following a bimodal growth cycle with a primary maximum in the spring and a secondary maximum in the fall (Thorne-Miller et al. 1983; Orth and Moore 1986). Shoot to root-rhizome (S:RR) biomass ratios range from 0.6-2.0 depending upon plant shoot growth characteristics and are usually

about 2.0 during maximum eelgrass production (Orth and Moore 1986). Average eelgrass net production ranges from 1.0-14.0 $\text{gdw} \times \text{m}^{-2} \times \text{day}^{-1}$ and also is a function of shoot growth (Thorne-Miller and Harlin 1984; Moore et al. in press). Although detached plants may be transported, most of the biomass is retained in the meadow as detritus and up to 70% of the total detritus in an eelgrass bed is derived from eelgrass organic matter (Kenworthy and Thayer 1984). Eelgrass detritus is rapidly depleted of dissolved organic carbon within days of deposition as sediment heterotrophic bacteria utilize nearly 25% of the eelgrass carbon (Kenworthy and Thayer 1984; Blum and Mills 1991). The resulting material creates a particulate organic carbon pool that provides substrate for further heterotrophic metabolic consumption (Kenworthy and Thayer 1984).

Sediment microbial NH_4^+ regeneration from organic detritus and animal excretion create interstitial and sediment bound (exchangeable) NH_4^+ pools (Iizumi et al. 1982; Kenworthy et al. 1982; Short 1983; Moriarty et al. 1985; Boon 1986; Boon et al. 1986; Short 1987; Dennison et al. 1987; Caffrey and Kemp 1990). Vegetated areas usually contain considerably more total NH_4^+ than adjacent unvegetated sediments (vegetated ca 250 μM vs unvegetated ca 100 μM) (Bulthuis and Woekerling 1981; Iizumi et al. 1982; Kenworthy et al. 1982; Blackburn and Henricksen 1983; Caffrey and Kemp 1990). Increased sediment organic content,

and therefore increased NH_4^+ , augments eelgrass production and the vegetated sediment pools turn over 2-3 times faster than those of unvegetated sediments due to plant nitrogen demand (Short 1981; Iizumi et al. 1982; Short 1983; Short and McRoy 1984; Boon et al. 1986; Caffrey and Kemp 1990).

Seagrass productivity and nutrient uptake influence sediment redox conditions and nutrient cycling and the plants serve as transformers of sediment inorganic nutrients to in situ organic detritus (Hopkinson and Schubauer 1984; Boon 1986; Short 1987; Caffrey and Kemp 1990). Eelgrass releases up to 10% of O_2 produced to the sediment rhizosphere during times of maximum growth (19-23°C) (Caffrey and Kemp 1991). This activity creates microhabitats of rapid inorganic nitrogen transformation (Hopkinson and Schubauer 1984; Reddy and Patrick 1989). Heterotrophic organic remineralization, nitrogen fixation, and dissimilatory nitrate reduction add to ammonium reserves while microbial and macrophytic assimilation, nitrification-denitrification, and diffusion-advection reduce ammonium supplies (Capone 1982; Boon et al. 1986; Caffrey and Kemp 1990).

Comparative uptake experiments between water column and sediment NO_3^- and NH_4^+ indicate sediment NH_4^+ to be the optimal nitrogenous form for seagrasses (Patriquin 1972; Iizumi and Hattori 1982; Short 1987). Plant NH_4^+ incorporation is energetically less costly than NO_3^- use and sediment NH_4^+ production rates and concentrations are much

greater than those of the water column (Short 1987). The shoot components can produce nitrogen uptake rates sufficient to meet plant nitrogen requirements given an enriched water column (Thursby and Harlin 1982; Short and McRoy 1984; Borum et al. 1989), but the sediment is the primary source of inorganic nitrogen for eelgrass (Short 1987). Shoot uptake is concentration dependent and appears to work as an "ammonium sponge" as rapid uptake during times of high availability has been observed (Thursby and Harlin 1982; Short and McRoy 1984). Thursby and Harlin (1982) demonstrated root NH_4^+ uptake to be inhibited by active leaf uptake. Short and McRoy (1984) refuted this conclusion by showing root-rhizome (RR) NH_4^+ uptake to be concentration dependent over a range of interstitial concentrations (≤ 240 μM) while the leaves simultaneously capitalize on sporadic ammonium pulses. It appears RR NH_4^+ uptake follows Michaelis-Menten kinetics up to saturation at approximately 100 μM (Iizumi and Hattori 1982; Thursby and Harlin 1982; Short and McRoy 1984; Dennison et al. 1987).

Studies have been conducted to investigate whether eelgrass production can be nitrogen limited (Bulthuis and Woelkerling 1981; Harlin and Thorne-Miller 1981; Kenworthy et al. 1982; Short 1987; Dennison et al. 1987; Zimmerman et al. 1987). Bulthuis and Woelkerling (1981), Harlin and Thorne-Miller (1981) and Short (1987) found positive growth response to nitrogen fertilization suggesting nitrogen

limitation, while Kenworthy et al. (1982), Dennison et al. (1987) and Zimmerman et al. (1987) reported no effect of high sediment nitrogen availability on plant production. Duarte (1990) suggested that seagrass whole plant tissue below $0.018 \text{ gN} \times \text{gdw}^{-1}$ is nitrogen limited. Eelgrass displays the widest range of nitrogen content (1.2- \rightarrow 5.0% N) for any seagrass worldwide (Duarte 1990). Determination of tissue nitrogen content depends upon both the seasonal physiological status and the plant part analyzed (Pirc and Wollenweber 1988; Borum et al. 1989).

Sediment sulfide levels $\geq 0.25 \text{ mM}$ can greatly inhibit NH_4^+ uptake by *Spartina alterniflora* (Bradley and Morris 1990). This could be the case with eelgrass. Pregnall et al. (1984) found that RR uptake of NH_4^+ can only occur during oxic rhizosphere conditions because the attachment of NH_4^+ to glutamate to form the amino acid glutamine requires ATP produced from the Krebs cycle. The levels of glutamate and therefore glutamine decrease markedly under anoxic conditions (Pregnall et al. 1984).

Competition for inorganic nitrogen resources with rhizosphere bacteria could be another factor affecting eelgrass nitrogen availability. Caffrey and Kemp (1990) conclude that this competition operates in freshwater sediments vegetated by *Potamogeton perfoliatus* but not necessarily in eelgrass beds because sediment nitrogen transformations (ammonification, nitrification,

denitrification/nitrate reduction) and plant growth processes are not tightly coupled. The role of internal plant translocation and reclamation of nitrogen reserves could explain in part the apparent discrepancy between sediment NH_4^+ abundance and plant response to nitrogen enrichment. Eelgrass transports nitrogen from mature and senescent tissues to growing tissues to meet N growth requirements (Iizumi and Hattori 1982; Borum et al. 1989). This mechanism is believed to be an adaptation to the temporal and spatial variability of external nitrogen resources in temperate marine ecosystems.

The specific biogeochemical nutrient transformations which occur at the rhizosphere interfaces and the abilities of seagrasses to use both sporadic external nitrogen supplies and internal reserves must be better understood before the question of nitrogen limited production can be resolved. Few studies have focused on nitrogen cycling and uptake in seagrass beds and no specific uptake experiments have been performed on Chesapeake Bay eelgrass where Orth (1977) reported plant response to nitrogen enriched fertilization. Investigations are needed on seasonal changes in regeneration and assimilation of sediment NH_4^+ , nutrient stocks and transformations for Chesapeake Bay seagrasses, and the overall functioning of vascular plants in biogeochemical cycling (Bulthuis and Woelkerling, 1981; Iizumi et al. 1982; Hopkinson and Schubauer 1984).

A particularly useful technique for identifying pathways of nitrogen exchange and transformation in natural systems is through the use of the stable isotope, ^{15}N (Harrison 1983). The movement of compounds labelled with ^{15}N between different inorganic and organic pools can be traced under experimental conditions. For nutrient uptake experiments, the isotope tracer method is more precise and sensitive than the spectrophotometric method of measuring external pool nutrient concentration decline over the experimental incubation time (Williams and Fisher 1985). In spectrophotometric experiments, there is uncertainty in attributing the disappearance of the nutrient to the activity of the organism in question as other factors (e.g. uptake and regeneration by extraneous microflora or fauna, adsorption of the nutrient to the experimental vessel) can affect the nutrient concentrations. The ^{15}N isotope has been employed in the study of agricultural plants and marine phytoplankton for many years but has been used sparsely for aquatic macrophyte nitrogen dynamics (Iizumi and Hattori 1982; Short and McRoy 1984; Williams and Fisher 1985; Boon et al. 1986; Borum et al. 1989).

The primary objectives of this project were to quantify sediment inorganic nitrogen stocks and plant characteristics and to measure root-rhizome $^{15}\text{NH}_4^+$ uptake rates over a range of concentrations for eelgrass plants of the lower Chesapeake Bay to obtain a better understanding of plant-

sediment nitrogen interactions in temperate estuarine systems.

C. PROJECT OBJECTIVES AND HYPOTHESES

The specific objectives of the proposed research were to:

1. Determine sediment inorganic nitrogen standing stocks (0-10cm) of an eelgrass meadow of the lower Chesapeake Bay at monthly and seasonal intervals.

H₀: Sediment NH₄⁺ and NO_x⁻ (NO₂⁻ + NO₃⁻) do not vary monthly.

2. Seasonally determine average eelgrass biomass and plant C:N ratios and estimate average seasonal nitrogen demand using growth data available in the literature.

H₀: Nitrogen demand does not vary seasonally.

3. Measure eelgrass root-rhizome ¹⁵NH₄⁺ uptake rates over a range of controlled, experimental nitrogen concentrations and incubation times to determine NH₄⁺ uptake kinetics.

H₀: Eelgrass RR ammonium uptake rates are independent of available concentrations.

H₀: Duration of incubation has no effect upon uptake kinetics.

D. METHODOLOGY

1. Justification

The development of this research project began with a familiarization of field conditions, experimental design, and laboratory analytical techniques through a series of trials and pilot studies. Site selection was based upon both past and ongoing research in a seagrass meadow known to be stable

over time. Techniques of meadow characterization (plants and sediments) were based upon past and present VIMS SAV-nutrient protocols and literature methods. The ammonium uptake design was developed following an intensive literature review, discussions with researchers active in the field, and laboratory studies to develop protocols for the processing of stable isotope samples using emission spectrometry. The NH_4^+ treatment concentrations were determined from the field data collected just prior to the experiments. The experimental incubation times were chosen as a compromise between hourly series and consideration of the labor intensive nature of stable isotope analysis. The project objectives were defined to complement and augment the VIMS SAV-Nutrient program particularly in an area where limited information and data were available.

2. Site Selection

The eelgrass meadow on the northern shoreline of the York River mouth (Virginia, USA) locally known as Guinea Marsh was chosen for study because of the extent of data available for the area and its close proximity to the VIMS laboratory and seagrass greenhouse (Fig. 1).

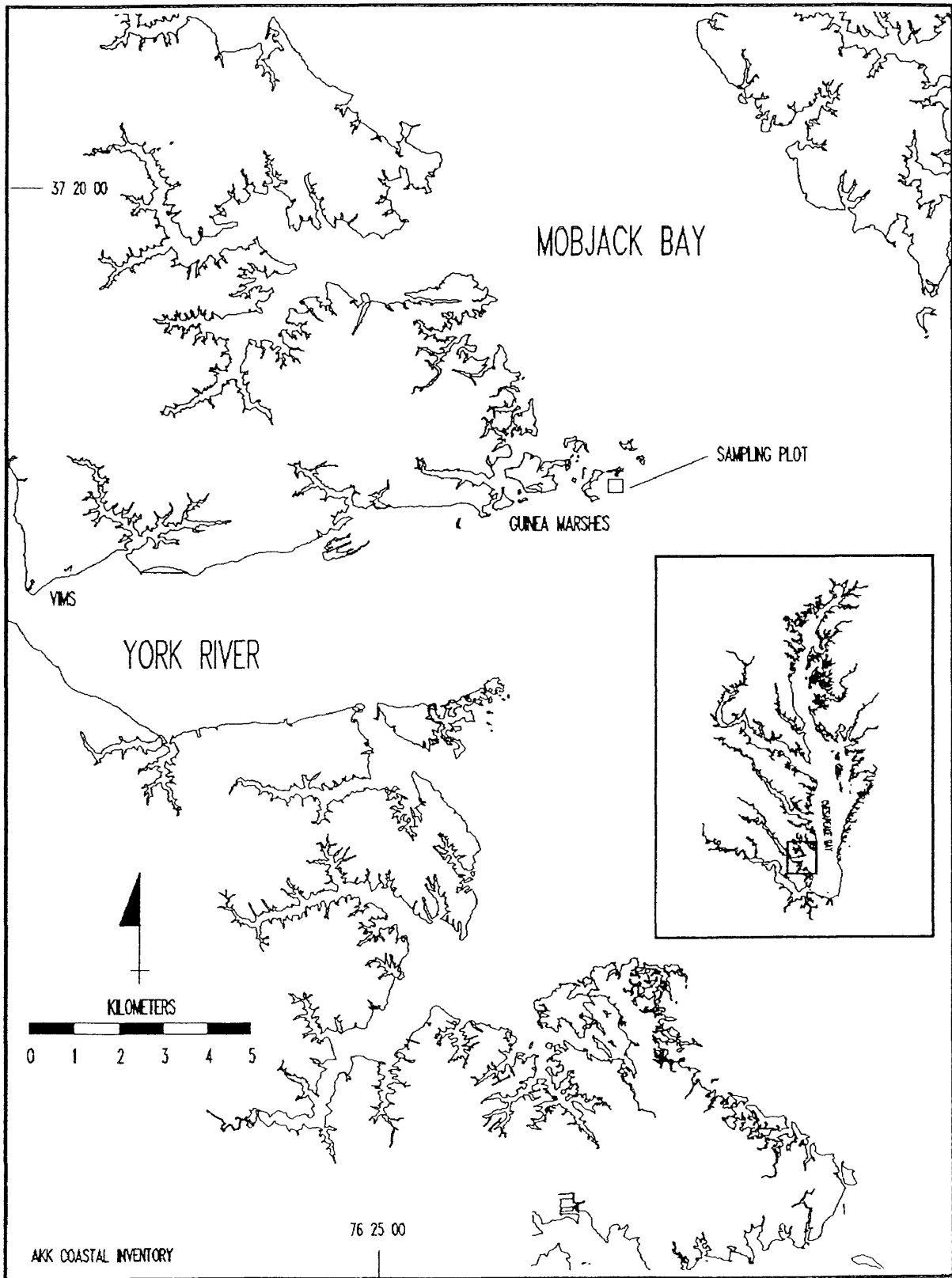
3. Sampling and Experimental Design

A 100 m² plot was established in the Guinea Marsh eelgrass meadow to provide a permanent sampling area for this study. The plot was located ca 500 m (ca 0.6 m @ mean low water) from the marsh shoreline because this area is the site of the continuing York River nearshore monitoring

program, is mid-way along past seagrass distribution and abundance transects, and is believed to be out of the direct effects of marsh influence. The bi-weekly shoal water survey provided incident and submarine irradiance, temperature (air and water), and salinity data for use in conjunction with similar physical data recorded during this study. This was done to acquire a more complete index of physical conditions over the sampling time span of this study. Total irradiance was measured at the water surface, 10 cm below the surface, and ca 10 cm off the bottom. K_d , the vertical attenuation coefficient, was calculated from these data. Approximately monthly for one year (June 1990-May 1991) the physical parameters and sediment and plant cores were sampled. Cores were not taken randomly due to the patchy eelgrass distribution during some times of the year and the sediment core sectioning problems discussed in the next section. Initially, samplings were performed at the end of the month but after inclement weather at the end of November, 1990 sampling occurred at the beginning of each month.

Samples consisted of six 2.22 cm ID sediment cores (3 for %water, %organic matter (OM), and sediment or bulk density (BD) and 3 for NH_4^+ and NO_x^- ($\text{NO}_3^- + \text{NO}_2^-$), all determined at 1 cm increments down to 10 cm) and 3 plant biomass cores (0.0205 m^2). Every fourth month (June, September, January, April) 12 sediment cores were taken, 6 for sediment properties and 6 for nutrients. This was done

Figure 1. Map view of Chesapeake Bay with sampling site on the northern edge of the York River mouth.



to determine the variation in the inorganic nitrogen concentration results in light of the spatial heterogeneity of the plants and nutrients. These larger samplings were performed 1-2 weeks prior to the commencement of the greenhouse $\text{RR } ^{15}\text{NH}_4^+$ uptake experiments (GHEX). Uptake experiments were performed 24 July 90, 2 November 90, 20 February 91, and 25 April 91. The first experiment (GHEX 1) became a pilot study as protocol was refined. The results of experiments GHEX 2 (autumn), GHEX 3 (winter), and GHEX 4 (spring) are reported in the following sections.

4. Sediment Characterization

A 10 cm coring depth was chosen to correspond with root-rhizome biomass distribution data reported in Wetzel (1981). Acrylic tubes (2.22 cm ID x 10 cm) were pushed into the sediment by hand directly adjacent to eelgrass plants. Ideally, the core would have been pushed over individual plants but this creates difficulties in easily sectioning the cores. The replicate cores were selected, capped at both ends, and stored on ice for transport back to the laboratory. In the laboratory the overlying water was siphoned from both sets of cores (bulk density and inorganic nutrients).

The bulk sediment cores were extruded from the acrylic tubes by laying the tube almost horizontal across a piece of aluminum foil and gently blowing on the bottom end of the core to remove the intact sediment without compressing or stretching. The sediment was then cut into 1 cm increments

and each increment was placed into a small, pre-combusted, aluminum weigh pan. The increments were then weighed wet and placed into a 60 °C drying oven. After at least 96 hrs the individual pans, each with a 1 cm sediment increment, were again weighed to determine dry weight. The pans and contents were then placed into a 500 °C combustion oven for 5 hrs and then weighed a final time. The water content (wet wt - dry wt/ wet wt) and organic content (dry wt - ash wt/ dry wt) were expressed as percentages. The sediment or bulk density was calculated from the wet weight and the volume of the 1 cm section.

The nutrient cores were extruded and sectioned similarly to the bulk sediment cores. In this case, the individual sections were cut and placed into tared, 50 ml centrifuge tubes and weighed wet. KCl (1 N) was used to extract the inorganic ion complexes from the individual sediment sections. Twenty-five mls of 1 N KCl were added, the tubes were shaken for 15 minutes, and then centrifuged at 3000 rpm for 10 minutes. Two 3.5 ml sub-samples of the supernatant were pipetted into 10 ml culture tubes and the total NH_4^+ concentration was determined spectrophotometrically using the phenolhypochlorite method against standards prepared with KCl and standard stock NH_4^+ solutions (Parsons et al. 1984). The remaining supernatant was decanted into a clean centrifuge tube and frozen for later determination of NO_x^- using the cadmium reduction technique (Parsons et al. 1984).

5. Plant Characterization

Three replicate biomass cores (BIO) were intentionally (not randomly) selected from groups of eelgrass plants using a 16.2 cm ID x 15 cm acrylic coring device. The coring device was placed over the plants and worked into the sediment until almost flush with the sediment surface. Adhering sediment was rinsed from the eelgrass through a large mesh (1 cm²) sieve and plants were placed in plastic bags and stored on ice in the dark for transport. In the laboratory, plant samples were rinsed with tap water to remove the remaining sediment, and sorted and cleaned to remove animals, shell fragments, worm tubes, and macroalgae. Plant samples were then patted dry with paper towels, weighed, and then weighed after drying at 60 °C for ca one week. Subsamples of the dried plants were ground to a powder with mortar and pestle and the percent by weight of carbon and nitrogen were determined on three replicates each of shoot and RR tissue with a Perkin-Elmer model 240B CHN elemental analyzer using acetanilide as the standard.

6. ¹⁵N-NH₄⁺ Root-Rhizome Uptake Experiments (GHEX)

York River water was delivered to a large sand filter and then dispensed into both a large volume holding tank and through a 50 um mesh into a header tank. From the header tank the water was then fed into the four experimental aquaria which each held 15 individual plant incubation chambers (Figure 2).

The day before an experiment ca 200 whole eelgrass

plants were dug by shovel from the sampling site. Sixty plants were selected according to the criteria: total leaf number= 3; rhizome segments= 7. Each plant was rinsed of adhering sediments by hand during the selection process using filtered river water and then sealed with silicon grease through a holed stopper into a numbered, opaque 125 ml polyethylene flask (Fig. 2). The RR portion was suspended in 140 ml of 20 ppt Instant Ocean® inside the flask while the shoot was bathed by incoming filtered water provided by the greenhouse seawater flow-through system. Fifteen replicate plant-flask units were assigned to each of four aquaria (Fig. 2). The plants were affixed in their incubation chambers and designated to the appropriate aquaria usually by 1600 hrs the day before the onset of an experiment to acclimate the plants. For GHEX-2 (Nov, 90) and GHEX-3 (Feb, 91) all plants in a particular tank received the same experimental treatment (RR chamber NH_4^+ concentration); 1) avg field conc, 0 % ^{15}N atom excess; 2) avg field conc, 90 % ^{15}N atom excess; 3) ca 1.5 x avg. field conc, 90 % ^{15}N atom excess; 4) ca 2.0 x avg field conc, 90 % ^{15}N atom excess. Because of this, the possibility for a tank effect disguised as a treatment effect existed. In GHEX-4 the plants were randomized according to tank (1-4), treatment ($^{14}\text{NH}_4^+-\text{C}$, $^{15}\text{NH}_4^+-1$, $^{15}\text{NH}_4^+-2$, or $^{15}\text{NH}_4^+-3$), and sampling time (0, 4, or 10 hours). Some dispersion was utilized to keep the number per tank equal. This provided a mostly randomized design and enabled analysis of possible

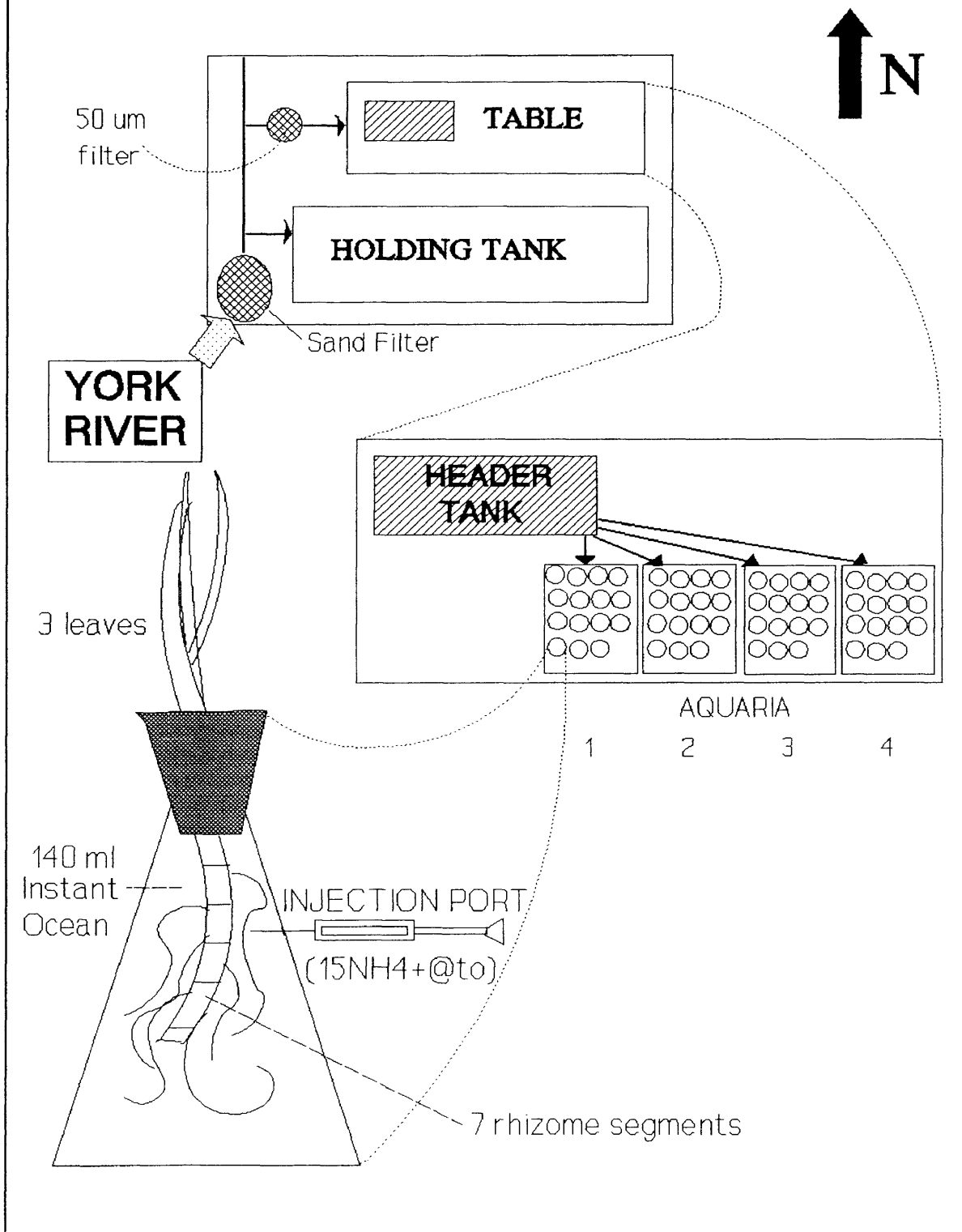
tank effects for the spring experiment. It is important to note that a block (tank) effect was not present in other eelgrass experiments in the VIMS greenhouse (Moore, pers comm).

Around daybreak on the day of the experiment each plant-flask unit was injected with a 1 ml treatment solution through a port located on the side of the flask (Fig. 2). At times 0 hrs, 4 hrs, and 10 hrs five plant units from each treatment were sampled. The whole plants were not rinsed and each plant was separated into its shoot and RR parts, placed into individually numbered weigh pans, and dried at 60 °C for ca one week. The treatment water in the flasks was decanted into labelled 50 ml centrifuge tubes and analyzed for total NH_4^+ using the phenolhypochlorite method (Parsons et al. 1984).

After drying, the shoot and RR were weighed and the RR were ground by mortar and pestle for analysis of ^{15}N content (atom % excess) using the methods of Fiedler and Proksch (1975) with a JASCO N-150 ^{15}N emission spectrometer. About 0.0005 g of dried, ground RR material was placed into an acid washed and pre-combusted 2.0 ml freeze drying ampoule. Pre-combusted micro-Dumas reagents (calcium oxide, cupric oxide, and Cuprox[®]) were added to each ampoule using a micro spatula. Two full scoops (ca 500 mg) of CaO and Cuprox[®] and one scoop (ca. 200 mg) of CuO were used. The ampoules were then evacuated to 2×10^{-4} torr using a high vacuum manifold and sealed by cutting with an oxypropane torch. The sealed

Figure 2. Schematic of experimental system used in Greenhouse Root/Rhizome $^{15}\text{NH}_4^+$ uptake Experiments (GHEX).

VIMS GREENHOUSE SCHEMATIC



samples were then combusted at 500 °C ca 12 hours and the % ¹⁵N atom excess was determined with the JASCO instrument. Standards were prepared from unenriched RR tissue freeze dried in the 2.0 ml ampoules with known amounts of (¹⁵NH₄⁺)₂SO₄³⁻ salt to create an enrichment range of 0.363-3.86% ¹⁵N atom excess. The standards were processed, combusted, and analyzed identically as the samples. Using the prepared standards, a standard curve was established using linear regression with the calculated (known) % ¹⁵N atom excess as the abscissa and the JASCO machine response as the ordinate. The resulting regression equation was solved for (x) and the machine response value, y, derived from the sample was incorporated into the equation to determine the actual % ¹⁵N atom excess of the unknown sample. The ammonium uptake rate was calculated according to the equation of Short and McRoy (1984) and Iizumi and Hattori (1982):

$$V = \frac{((\%^{15}\text{N plant})(\% \text{ N RR})(1 \times 10^6))}{((\%^{15}\text{N } t_0 \text{ pool})(14 \text{ gN})(\text{time}))}$$

Where:

V= uptake rate (umole N x gdw RR⁻¹ x hr⁻¹)
¹⁵N plant= atom excess at time-final
% N RR= gN x gdw RR⁻¹
umoles N x gN⁻¹= 1 x 10⁶/ 14= 71428.6
¹⁵N t₀ pool= atom excess of pool at time-0
time= time of incubation in hours

7. Statistical Methods

The 11 monthly averages of %organic matter (OM), NH₄⁺ concentration (uM), NO_x⁻ concentration (uM), plant biomass

(BIO), C:N of the shoot material (CNS), and C:N of the root-rhizome (CNRR) were analyzed using one-way analysis of variance (ANOVA; $n=3$) (Zar 1984; Wilkinson 1990). When the ANOVA was significant ($\alpha \leq 0.05$), Tukey's *a posteriori* multiple comparison test was used to differentiate the monthly means (Wilkinson 1990). Relationships among K_d , water temperature (TEMP), salinity (SAL), NH_4^+ , NO_x^- , BIO, CNS, and CNRR were investigated using a Pearson correlation matrix with associated probabilities and linear regression models (Zar 1984; Wilkinson 1990).

The minimum, maximum, and mean uptake rates over all NH_4^+ treatments ($n_{4\text{hr}}=15$; $n_{10\text{hr}}=15$; $N_{\text{total}}=30$) were calculated for each RR uptake experiment (Wilkinson 1990). The 4 hr and 10 hr means within each experiment were compared using a t-test. Within each incubation time, the mean NH_4^+ uptake rate per treatment were analyzed by one-way ANOVA (3 treatments, $n=5$). When significant ($\alpha \leq 0.05$), Tukey's *a posteriori* multiple comparison was used to differentiate the treatment means (Zar 1984). A Michaelis-Menten model was used to estimate the kinetic parameters of V_{max} (maximum uptake rate) and K_s (substrate concentration where $V=(0.5)(V_{\text{max}})$) through multiple iterations designed to minimize the variance between the non-linear model and the actual data (Wilkinson 1990).

III. RESULTS AND DISCUSSION

A. RESULTS

1. Field data (June 1990-May 1991)

Air and water temperature, vertical light attenuation (K_d), and salinity presented are monthly means from the combined data of the water column survey and this study. Air and water temperature were maximum in August (27.0 and 26.8 °C, respectively) and minimum in December and February (5.0 and 5.8 °C) (Table 1). Salinity showed little variation with extremes occurring in June and December (16.1 and 21.0 psu). K_d , which ranged 0.54-1.66 m^{-1} , was maximum in June and minimum in April and averaged 1.12 m^{-1} over the entire 11 months of sampling (Table 1). Sediment water content (%water; 21.1-24.6) and bulk density (BD; 2.06-2.24) varied little throughout the sampling period (Table 1). Sediment organic matter (OM) was greatest in June (1.64%) and least in September (0.70%) (Table 1). Levels of OM in June, July and October were similar ($p \geq 0.20$) and were significantly greater than all other months ($p \leq 0.03$). October was statistically similar to August, December, February, and May ($p \geq 0.10$) while September, December, January, February, March, and April were all similar ($p \geq 0.5$) (Table 1).

Figure 3 contains the monthly vertical profiles of sediment NH_4^+ and NO_x^- . These data were derived from the total extractable pools and were not divided into absorbed vs interstitial. It is assumed this total pool (0-10 cm) is

potentially available for plant uptake (Wetzel 1981; Short 1981). Maximum NH_4^+ occurred at 3-6 cm except in July, September, and January when it occurred deeper (8-10 cm). Minimum NH_4^+ was in the 0-1 cm section except in March, April, and May when it was 8-10 cm in depth. Mean monthly NH_4^+ (0-10 cm) was greatest in June at 349.3 μM ($p \leq 0.05$) and least in February and March (50.0-60.0 μM , $p \leq 0.05$) with all other months being statistically similar (Table 2 and Figure 4). Sediment NO_x^- showed much variability in vertical location of maximum and minimum concentrations (Figure 3). October NO_x^- was significantly greater than all other months (91.0 μM , $p \leq 0.001$) (Table 2 and Figure 4). September and December represented the middle range of values (ca 43.0 μM) and differed from both October and the minima recorded in February and April (ca 17.0 μM , $p \leq 0.05$). Because sediment NH_4^+ and NO_x^- did in fact vary monthly the null hypothesis of objective #1, section C was rejected.

Table 3 and Figure 5 show the estimates of eelgrass biomass and C:N measured over the sampling period. Maximum biomass was attained in May and equalled 554.1 $\text{gdw} \times \text{m}^{-2}$ while lowest biomass was recorded for February at 141.1 $\text{gdw} \times \text{m}^{-2}$ (Table 3). June differed significantly from all other months ($p \leq 0.01$) except for July, April, and May ($p \geq 0.30$). September, December, January, February, and March were all statistically similar ($p \geq 0.40$). Shoot %carbon did not display any patterns and was fairly constant over the entire year (Table 3). Shoot %nitrogen also did not show

any significant patterns but it is important to note that values $\leq 1.8\%$ were never measured (Table 3). Shoot molar C:N ratios (CNS) were greatest in June at 21.1 and least in February at 12.3 (Table 3 and Figure 5). June, July, and August were statistically different from the other months ($p \leq 0.02$) representing the high end of the range. December, January, and February were significantly different than other months and represented the minimal values ($p \leq 0.005$). RR %carbon did not display any distinct patterns but %nitrogen was greatest during the least active periods for the plants (July, January, February, and March) (Table 3). RR %nitrogen frequently fell below 1.8% by dry weight. Root-rhizome C:N molar ratios (CNRR) were greatest in April (27.6) and lowest in March at 15.6 (Table 3 and Figure 5). April CNRR was significantly different than August, January, February, and March ($p \leq 0.04$) while September and December differed from March ($p \leq 0.02$). The June determination of RR C:N was believed to be spurious due to the probable contamination of the analytical samples by inclusion of a mixture of plant tissues (shoot, RR, living, dead) and was omitted from the statistical testing.

Table 4 is a correlation matrix of all variables measured at the field site from June 1990-May 1991. Temperature was positively correlated with salinity ($r = 0.55$), NH_4^+ ($r = 0.46$), OM ($r = 0.55$), BIO ($r = 0.52$), CNS ($r = 0.89$), and CNRR ($r = 0.38$). Sediment NH_4^+ was positively correlated to OM ($r = 0.35$) and CNS ($r = 0.39$). OM was

positively correlated to BIO ($r= 0.40$) and CNS ($r= 0.67$) while BIO and CNS were also positively correlated ($r= 0.50$). Figure 6 is a representation of these significant relationships. Linear regression models of these variables supplied the data for Table 5 and Figure 7a-7e. Figure 7a and 7b suggest a functional dependence of CNS upon both BIO and NH_4^+ . Figure 7c and 7d suggest functional dependence of OM upon both BIO and CNS, but OM positively influenced sediment NH_4^+ (Figure 9e) (Zar 1984). Significant relationships between NH_4^+ and BIO were not demonstrated.

Table 1. Field site physical characteristics and sediment properties of eelgrass bed of lower York River (Summer 1990-Spring 1991; n=3, K_d =vertical light attenuation coefficient, 0-10cm mean±sd, Bulk Density=g x cm⁻³). ANOVA was performed on %organic matter only; Superscripts a, b, c, and d denote statistical similarity (Tukey's a posteriori multiple comparison; $\alpha \leq 0.05$).

MONTH	TEMP(C)*		SAL*	K_d^*	%H ₂ O		%ORGANIC		BD
	air	H ₂ O			psu	m ⁻¹	mean±sd	mean±sd	
JUNE	24.8	23.4	16.1	1.66	23.1±1.00	1.64±0.18 ^a	2.09±0.02		
JULY	26.8	26.5	19.7	1.34	22.6±0.88	1.62±0.06 ^a	2.06±0.06		
AUG	27.0	26.8	19.2	1.23	24.6±2.28	1.18±0.26 ^b	2.12±0.04		
SEPT	24.3	21.2	18.5	1.58	23.2±0.98	0.70±0.10 ^d	2.24±0.04		
OCT	22.3	19.5	18.5	0.98	22.9±0.55	1.31±0.21 ^{abc}	2.10±0.02		
DEC	6.8	8.5	21.0	1.21	21.3±0.64	0.96±0.12 ^{cd}	2.15±0.04		
JAN	6.9	6.4	16.4	1.13	21.1±0.51	0.86±0.07 ^d	2.18±0.04		
FEB	5.0	5.8	16.2	0.88	21.7±1.42	0.94±0.20 ^{cd}	2.11±0.05		
MAR	9.8	8.6	17.3	0.74	21.9±1.14	0.84±0.09 ^d	2.16±0.06		
APR	13.8	14.1	16.8	0.54	21.8±0.22	0.80±0.03 ^d	2.14±0.06		
MAY	25.7	22.3	17.9	0.99	24.6±1.07	1.17±0.12 ^{bc}	2.06±0.06		

*=value represents mean of multiple samplings from bi-weekly water column survey and this study, all other values are means of samples taken from this study.

Figure 3. Monthly vertical profiles of sediment NH_4^+ and NO_x^- ($\text{NO}_3^- + \text{NO}_2^-$) concentrations (μM).

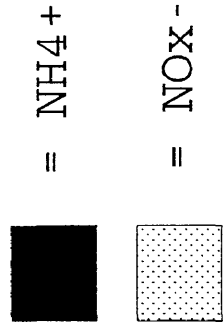
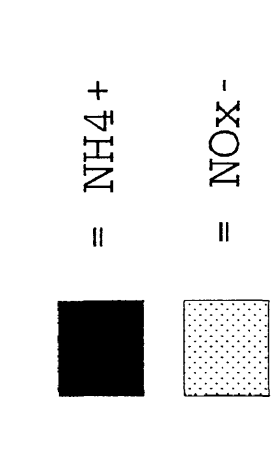
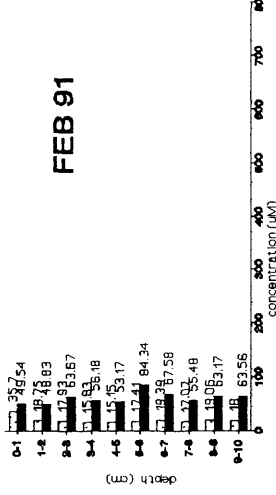
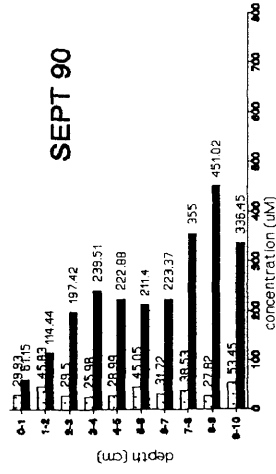
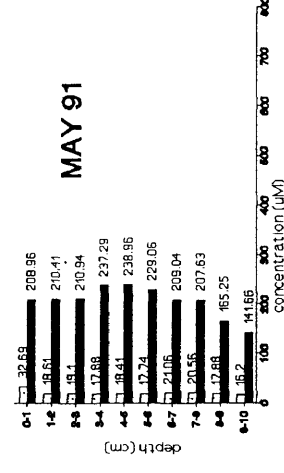
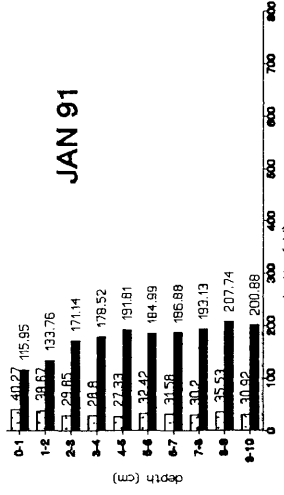
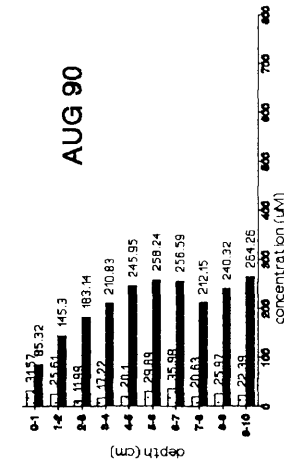
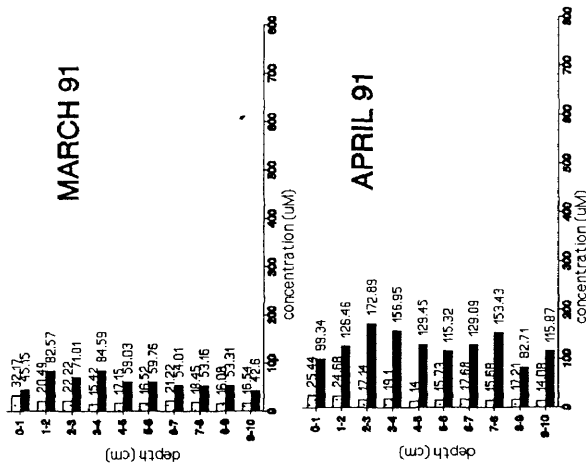
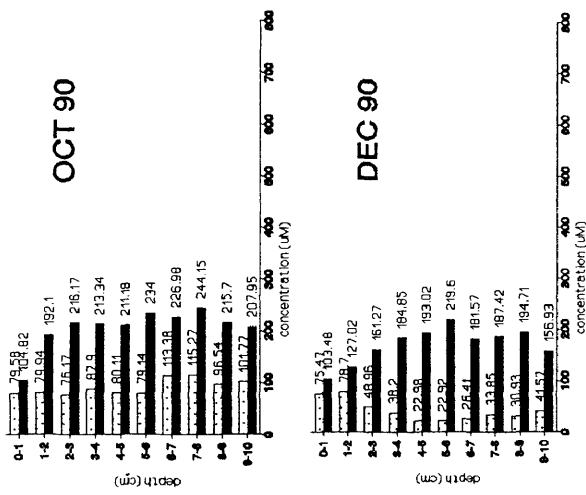
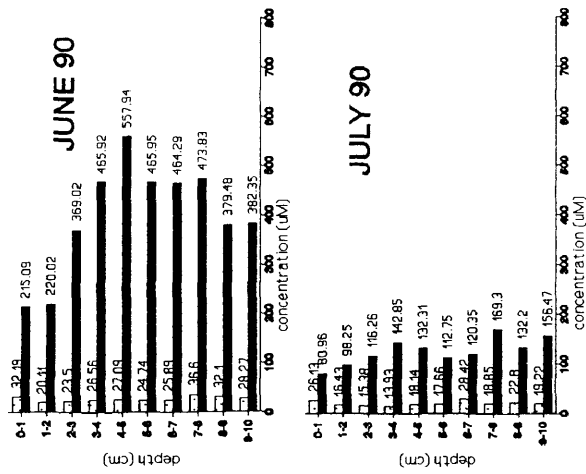
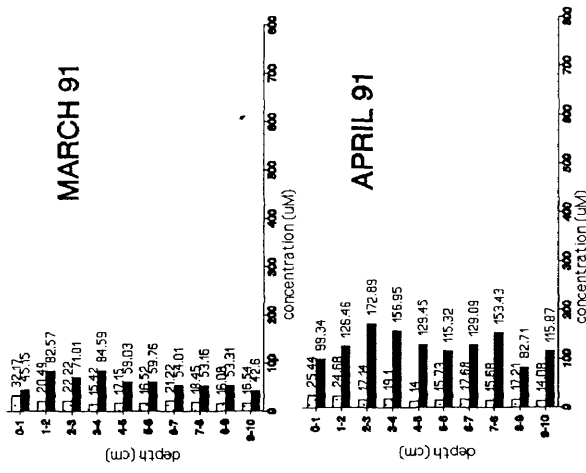
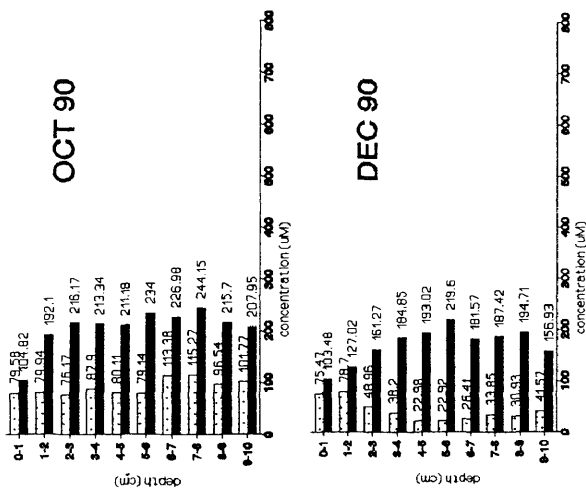
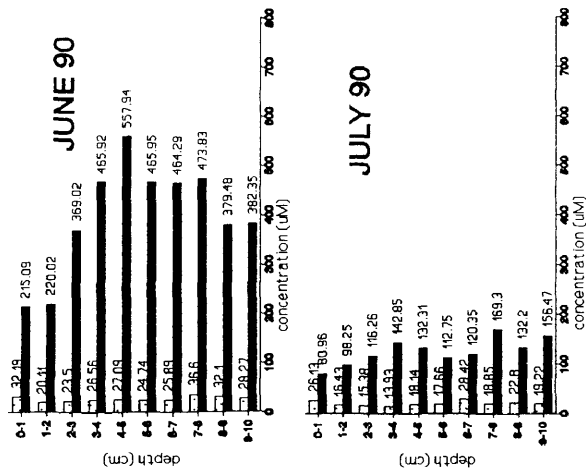


Table 2. Eelgrass sediment inorganic nitrogen (NH_4^+ and NO_x^-) concentrations (μM , 0-10 cm) (Summer 1990-Spring 1991), $n=3$, mean \pm sd. Superscripts a, b, and c denote statistical similarity (one-way ANOVA; Tukey's test).

	NH_4^+	NO_x^-
MONTH	mean \pm sd	mean \pm sd
JUNE	349.3 \pm 78.4 ^a	26.6 \pm 1.7 ^a
JULY	146.6 \pm 64.4 ^b	19.7 \pm 3.8 ^a
AUG	210.2 \pm 40.3 ^b	24.1 \pm 2.5 ^a
SEPT	188.7 \pm 44.4 ^b	44.1 \pm 17.7 ^{ab}
OCT	203.9 \pm 32.2 ^b	91.0 \pm 6.8
DEC	171.5 \pm 22.7 ^b	42.0 \pm 14.7 ^{ab}
JAN	174.8 \pm 82.0 ^b	34.4 \pm 1.0 ^{ab}
FEB	60.6 \pm 10.7 ^c	19.4 \pm 1.5 ^{ac}
MAR	51.2 \pm 23.2 ^c	21.8 \pm 5.3 ^{ac}
APR	109.4 \pm 45.7 ^b	15.4 \pm 1.4 ^{ac}
MAY	205.9 \pm 20.8 ^b	21.3 \pm 2.6 ^{ac}

Figure 4. Mean monthly sediment NH_4^+ and NO_x^- (0-10 cm; μM). Value shown is mean of 3 individual core averages (0-10 cm) calculated for each month.

SEDIMENT INORGANIC NITROGEN

June90-May91 (0-10cm means)

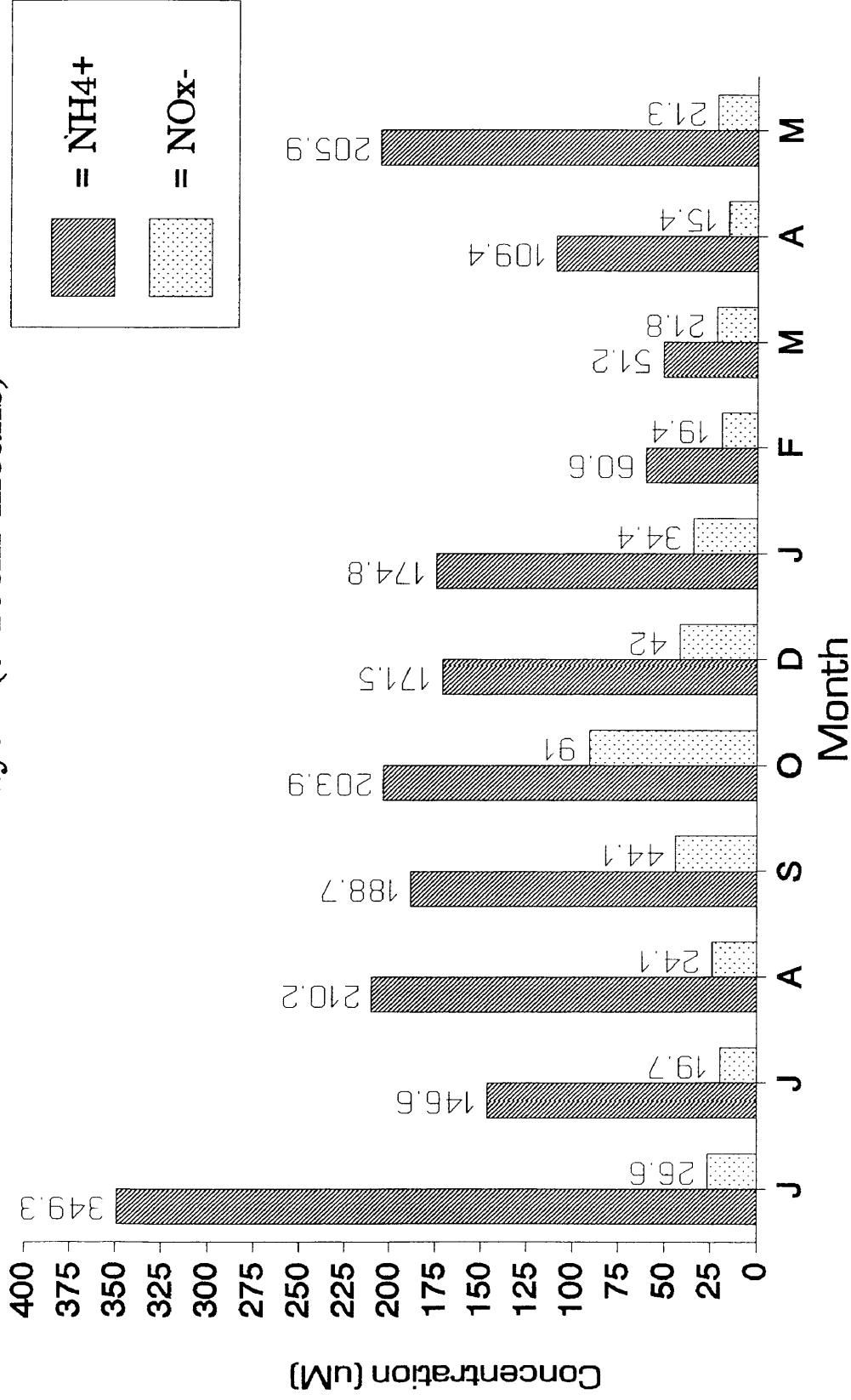


Table 3. Eelgrass growth characteristics (Summer 1990-Spring 1991, n=3, mean±sd; biomass= gdw x m⁻²; %C and %N by dry weight; C:N= molar ratio of Shoot and Root-Rhizome). Superscripts a, b, and c denote statistical similarity (one-way ANOVA; Tukey's test). (*) n=2.

Mon	BIOMASS		SHOOT		RR		C:N-S		C:N-RR	
	mean±sd	%C	%N	%C	%N	mean±sd	mean±sd	mean±sd	mean±sd	
JUN	511.4±112 ^a	38.7	1.84	29.4	1.89	21.1±0.1 ^a	15.6±0.7			
JUL	444.4±147 ^{ab}	38.0	1.82	37.5	1.74	20.8±0.1 ^a	22.0±3.8 ^a			
AUG	219.5±85.0 ^{bc}	38.8	1.98	26.8	1.38	19.6±0.1 ^a	19.4±1.4 ^a			
SEP	184.7±28.3 ^c	36.5	2.19	34.5	1.31	16.6±0.4 ^b	26.3±0.6 ^{ab}			
OCT	271.4±90.6 ^b	36.9	2.40	29.0	1.25	15.4±1.2 ^{bc}	23.3±0.8 ^{ab}			
DEC	169.8±32.4 ^c	33.7	2.51	26.1	1.07	13.5±1.0 ^c	24.6±2.9 ^{ab}			
JAN	*157.8±76.2 ^c	30.9	2.37	32.9	1.83	13.1±0.9 ^c	18.1±1.3 ^{ac}			
FEB	141.1±34.1 ^c	40.7	3.32	33.2	1.94	12.3±0.7 ^c	17.5±2.8 ^{ac}			
MAR	212.7±27.3 ^b	40.7	2.80	28.5	1.83	14.5±0.5 ^{bc}	15.6±0.8 ^{ac}			
APR	343.4±63.4 ^{ab}	36.6	2.37	31.4	1.19	15.5±1.3 ^b	27.6±6.0 ^{ab}			
MAY	554.1±104 ^{ab}	38.5	2.30	27.2	1.09	16.8±0.2 ^b	25.2±1.8 ^{ab}			

Figure 5. Plant biomass and shoot and root-rhizome molar C:N ratios (see Table 3).

Mean Monthly Plant Biomass and C:N Ratios June 90-May 91

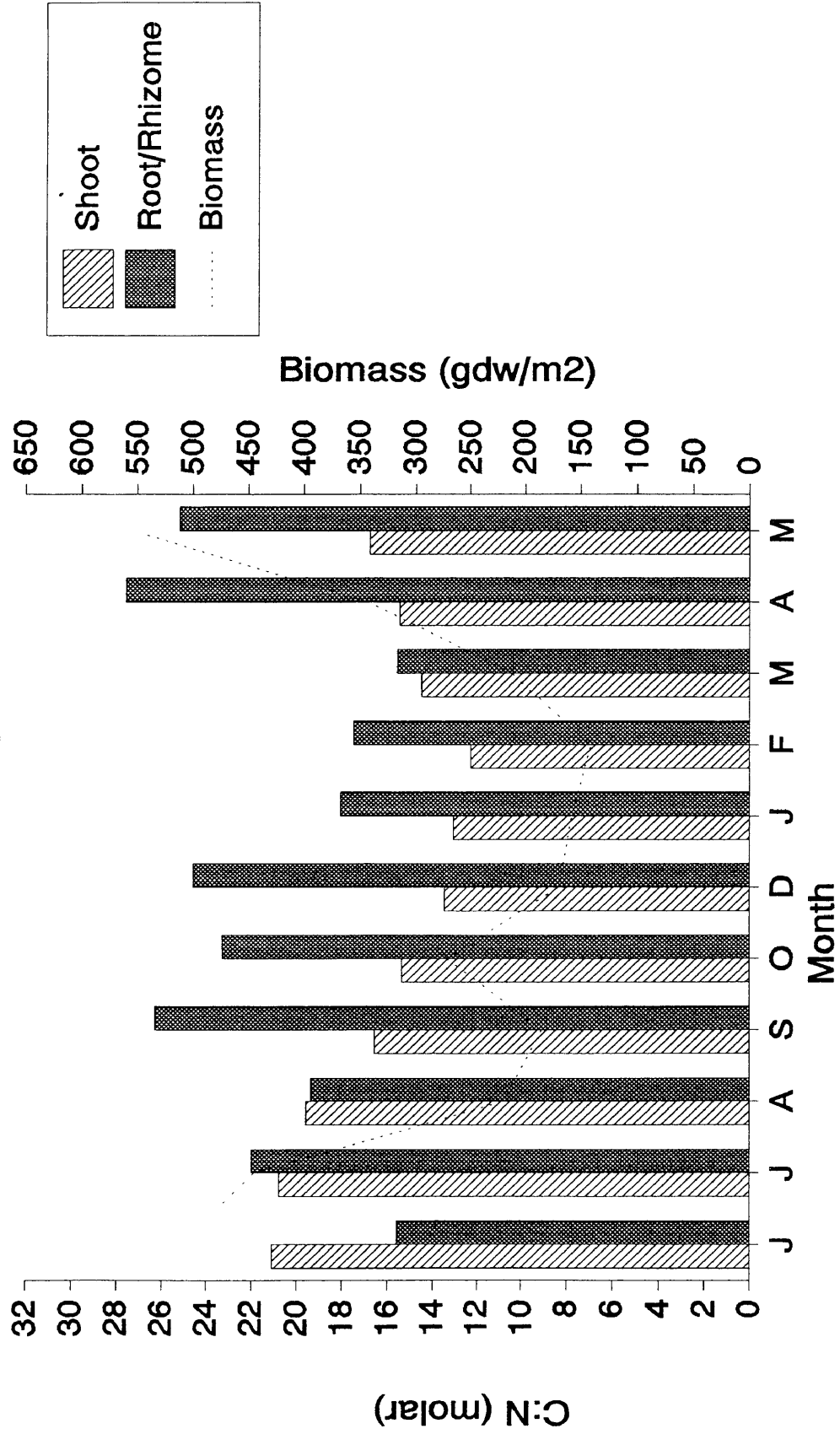


Table 4. Correlation matrix for field variables measured from June 1990-May 1991 (TEMP=water temperature, K_d =vertical light attenuation coefficient, SAL=salinity, NH_4^+ =sediment ammonium, NO_x^- =sediment nitrite + nitrate, OM=sediment organic matter, BIO=plant biomass, CNS=C:N ratio of Shoot, CNRR=C:N ratio of Root-Rhizome; n=26; table entry=correlation coefficient; $p < 0.10^*$, $p < 0.05^{**}$, $p < 0.01^{***}$).

	TEMP	K_d	SAL	NH_4^+	NO_x^-	OM	BIO	CNS
K_d	0.357*							
SAL	0.553***	0.492**						
NH_4^+	0.457**	0.234	0.380*					
NO_x^-	0.096	0.207	0.263	0.270				
OM	0.552***	0.093	0.393**	0.352*	-0.069			
BIO	0.524***	-0.069	0.231	0.030	-0.272	0.399**		
CNS	0.892***	0.181	0.478**	0.393**	-0.181	0.668***	0.496***	
CNRR	0.376*	-0.133	0.187	0.227	0.131	-0.025	0.415**	0.229

Figure 6. Eelgrass meadow correlative field variables.
Organic matter values= (calculated mean %)(10).

EELGRASS MEADOW DATA

June90-May91

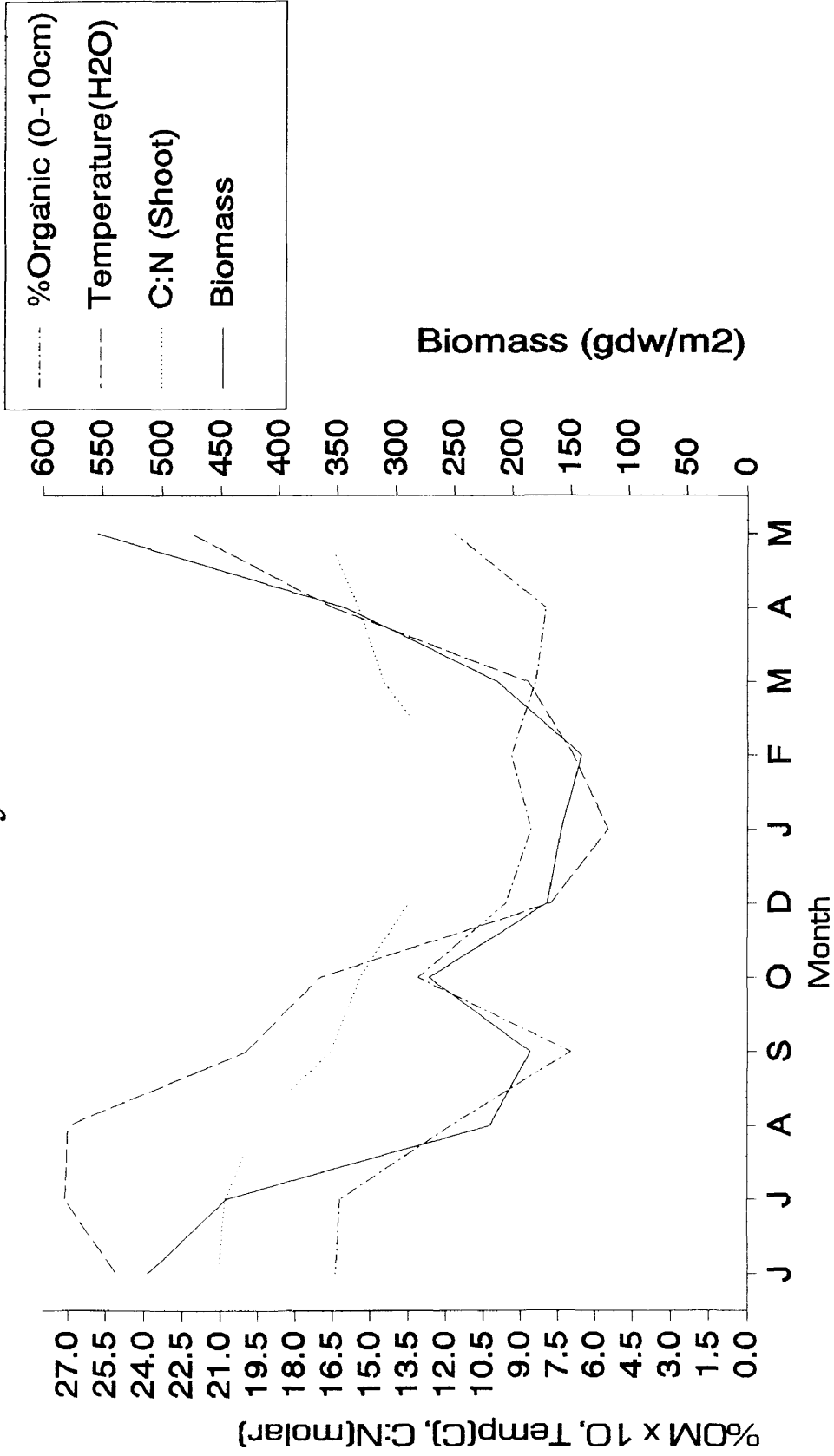
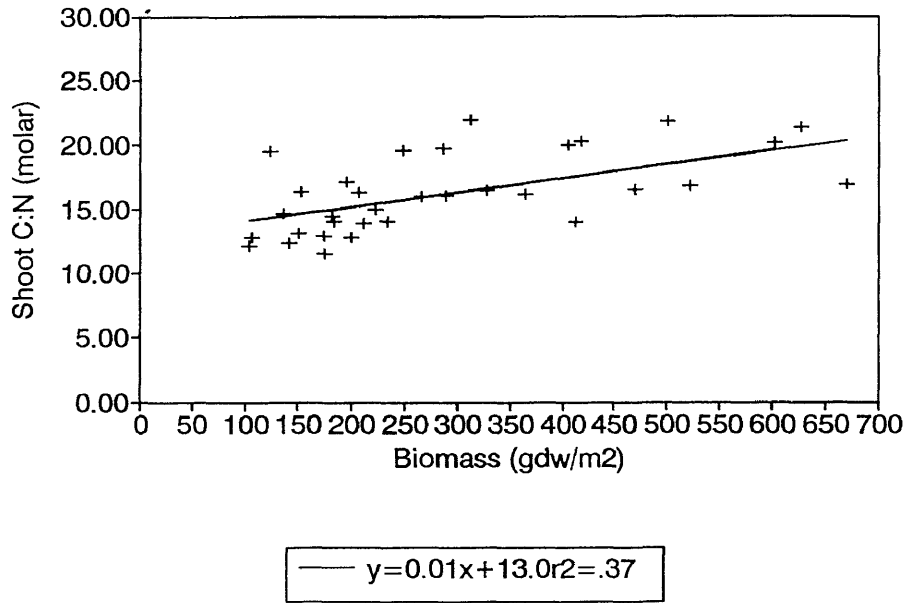


Table 5. Regression models between selected field variables (Summer 1990-Spring 1991, OM=sediment organic matter, BIO=plant biomass, CNS=C:N of plant shoots, NH_4^+ =sediment ammonium, n=number of paired samples, r^2 =regression coefficient, p= probability of significant slope).

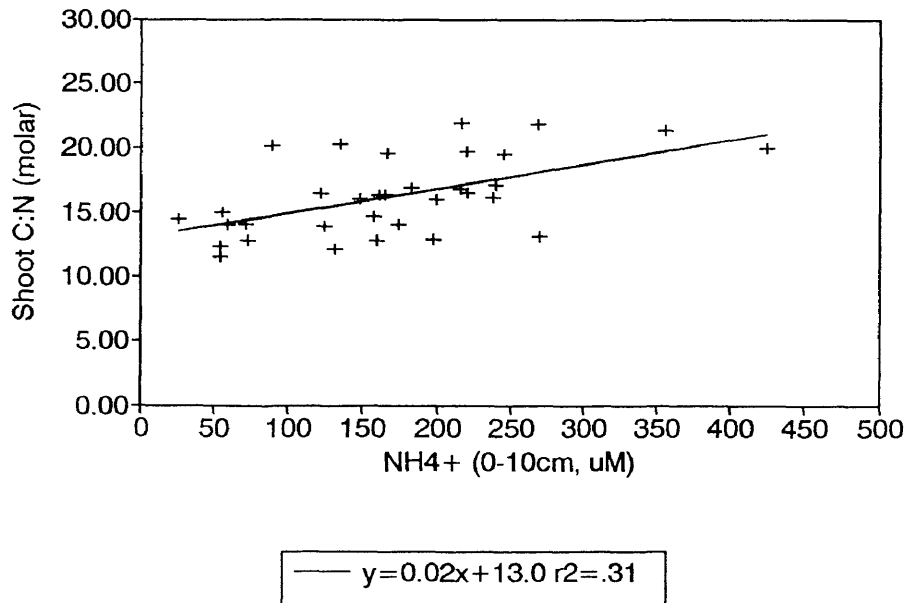
RELATIONSHIP y vs x	n	LINEAR REGRESSION	r^2	p (\leq)
CNS vs BIO	33	$y=0.011x+13.0$	0.366	0.001
OM vs BIO	33	$y=0.001x+0.751$	0.328	0.001
OM vs CNS	33	$y=0.078x-0.172$	0.497	0.001
NH_4^+ vs OM	33	$y=141.0x+16.16$	0.283	0.002
CNS vs NH_4^+	33	$y=0.019x+13.0$	0.313	0.002

Figure 7. Regression models between selected field
(a-e) variables. Data points represent all samples
taken over 11 months (June 90-May 91; n=3).

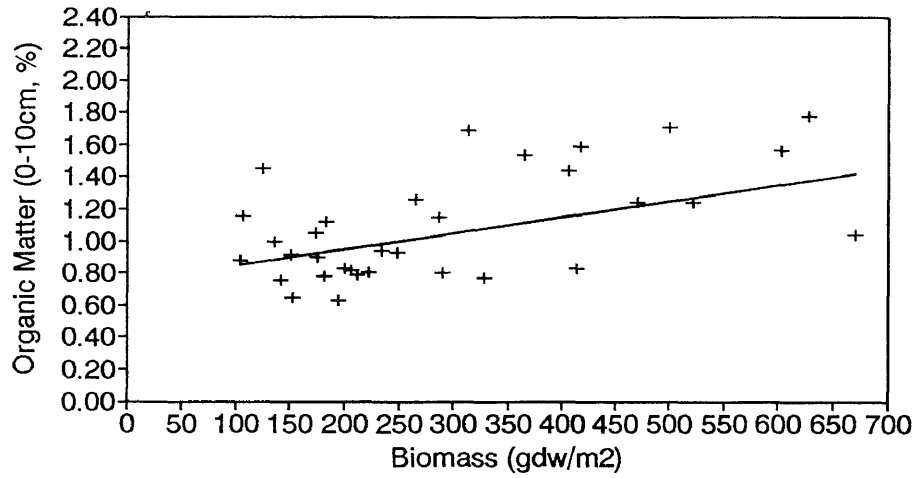
(7a) SHOOT C:N vs PLANT BIOMASS
June90-May91



(7b) SHOOT C:N vs SEDIMENT NH₄⁺
June90-May91

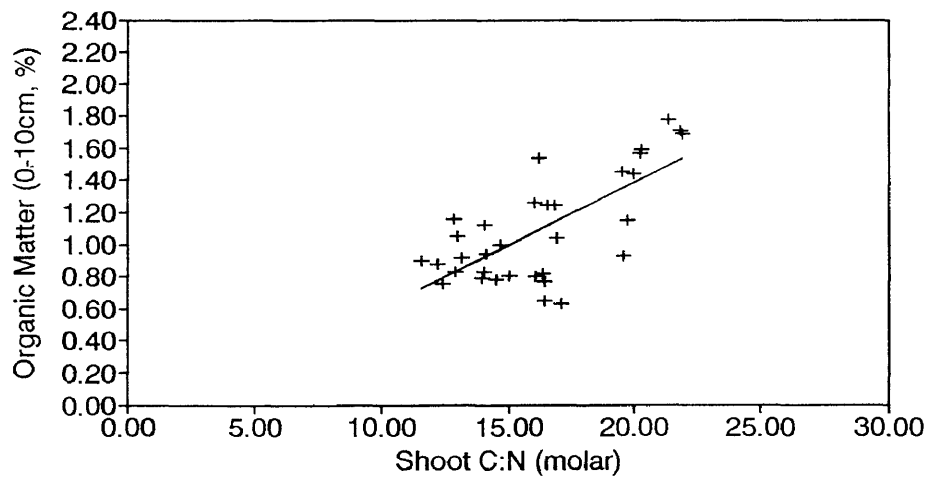


(7c) SEDIMENT % ORGANIC vs PLANT BIOMASS
June90-May91



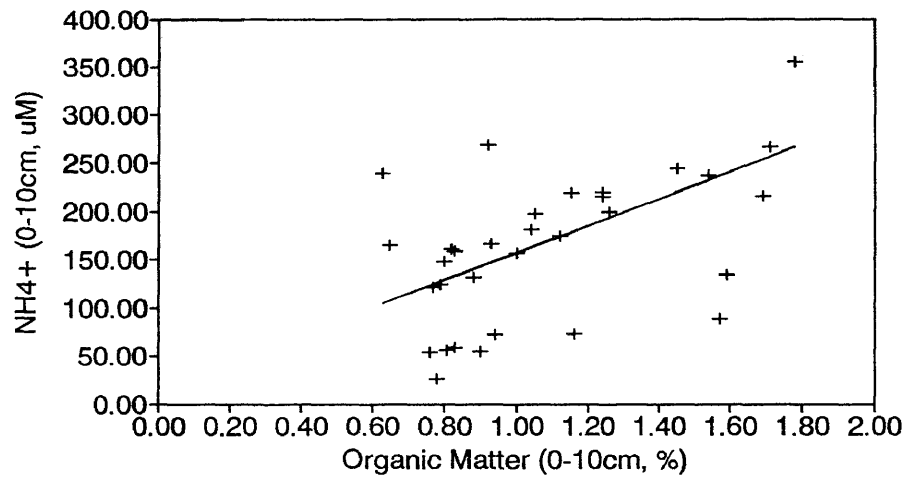
$y=0.001x+0.75, r^2=.33$

(7d) SEDIMENT % ORGANIC vs SHOOT C:N
June90-May91



$y=0.08x-0.17, r^2=0.5$

(7e) SEDIMENT NH₄⁺ vs ORGANIC MATTER
June90-May91



$y = 141x + 16.2$ $r^2 = 0.28$

2. Uptake Experiments (GHEX 2= 2 Nov 90, GHEX 3= 20 Feb 91, GHEX 4= 25 Apr 91)

Table 6 and Figure 8 provide the experimental conditions during each of the GHEX studies. In each experiment the thermistor and light sensor were placed in tank #2 (Figure 2) and all times reported are eastern standard time.

In GHEX 2 irradiance ranged 96.5-464.6 ($\mu\text{Einstein}$ s) $\mu\text{E} \times \text{m}^{-2} \times \text{sec}^{-1}$, averaged 224.0 $\mu\text{E} \times \text{m}^{-2} \times \text{sec}^{-1}$, and peaked ca 1300 hrs at 464.6 $\mu\text{E} \times \text{m}^{-2} \times \text{sec}^{-1}$ while temperature ranged 15.0-19.8 °C, averaged 18.1 °C, and peaked ca 1530 hrs at 19.8 °C (Figure 8a). These conditions are considered to be optimal for eelgrass growth (Wetzel and Penhale 1983). Uptake rates were greater over the initial 4 hrs of incubation than for 10 hrs in treatments (T1, T2, T3) 2 and 3 but were greater over the longer isotope exposure in T1 (Table 7 and Figure 9a). The mean 4 hr uptake rate averaged over all 3 treatments, 3.16 $\mu\text{mole N} \times \text{gdw RR}^{-1} \times \text{hr}^{-1}$, was significantly greater than the 10 hr average (2.35 $\mu\text{mole N} \times \text{gdw RR}^{-1} \times \text{hr}^{-1}$) ($p \leq 0.02$) (Table 8). Uptake rates ranged 0.78-5.02 $\mu\text{mole N} \times \text{gdw RR}^{-1} \times \text{hr}^{-1}$ for all plant incubation chambers ($n_{4\text{hr}} = 15$, $n_{10\text{hr}} = 15$; $N=30$) and averaged 2.75 $\mu\text{moles N} \times \text{gdw RR}^{-1} \times \text{hr}^{-1}$ (Table 8). Uptake of $^{15}\text{NH}_4^+$ was shown to be concentration dependent for the 4 hr interval as the T1 average rate was significantly different from T2 ($p \leq 0.02$) and T3 ($p \leq 0.01$) but appeared to saturate near 100 μM in the 10 hr incubation as treatments were determined to be

statistically similar ($p \geq 0.87$) (Table 7 and Figure 9b). The Michaelis-Menten kinetic parameters of maximum uptake velocity (V_{\max}) and the half saturation concentration (K_s , where $V = 0.5 * V_{\max}$) were estimated for the 4 and 10 hr incubations. The 4 hr plants displayed a $V_{\max} = 12.5$ umole N x gdw RR⁻¹ x hr⁻¹ and a $K_s = 462$ uM NH₄⁺ (Table 9). The 10 hr incubation had $V_{\max} = 2.58$ umole N x gdw RR⁻¹ x hr⁻¹ and $K_s = 14.1$ uM NH₄⁺. GHEX 2 involved the highest NH₄⁺ concentrations (Table 7), but as in GHEX 3 and GHEX 4 the NH₄⁺ concentrations used were representative of field conditions at the time of the experiment (Figure 4: October = GHEX 2, February = GHEX 3, April = GHEX 4).

In GHEX 3 irradiance ranged 50.3–201.0 uE x m⁻² x sec⁻¹, averaged 77.4 uE x m⁻² x sec⁻¹, and peaked ca 1300 hrs at 201.0 uE x m⁻² x sec⁻¹ (Figure 8b). Temperature varied less (10.1–11.2 °C) and averaged 10.5 °C. Four hour uptake rates were greater than 10 hr for all 3 treatments (Table 7 and Figure 9c). The mean 4 hr uptake rate averaged over all 3 treatments (2.13 umole N x gdw RR⁻¹ x hr⁻¹) was significantly greater than the 10 hr average (1.63 umole N x gdw RR⁻¹ x hr⁻¹) ($p \leq 0.03$) (Table 8). Uptake rate ranged 0.73–3.33 umole N x gdw RR⁻¹ x hr⁻¹ and averaged 1.88 umole N x gdw RR⁻¹ x hr⁻¹ (Table 8). Uptake of ¹⁵NH₄⁺ was concentration dependent in the 4 hr incubation as the T1 mean rate differed from T3 ($p \leq 0.05$) but not from T2 ($p \geq 0.40$) (Table 7 and Figure 9d). NH₄⁺ concentration did not significantly

influence uptake rates for the 10 hr isotope exposure ($p \geq 0.08$). In the 4 hr incubation $V_{\max} = 3.29$ and $K_s = 23.4$ while in the 10 hr $V_{\max} = 2.43$ and $K_s = 21.0$ (Table 9).

In GHEX 4 irradiance ranged 173.3-634.3 $\mu\text{E} \times \text{m}^{-2} \times \text{sec}^{-1}$, averaged 374.1 $\mu\text{E} \times \text{m}^{-2} \times \text{sec}^{-1}$, and peaked ca 1300 hrs at 634.3 $\mu\text{E} \times \text{m}^{-2} \times \text{sec}^{-1}$ (Table 6 and Figure 8c). Temperature ranged 13.4-18.9 °C, averaged 17.3 °C, and the experimental conditions were more optimal than in GHEX 2 (Table 6). The 4 hr uptake rates were greater than those of the 10 hr incubation for all treatments (Table 7 and Figure 9e). The mean 4 hr uptake rate averaged over all 3 treatments (2.14 $\mu\text{mole N} \times \text{gdw RR}^{-1} \times \text{hr}^{-1}$) was significantly greater than the mean 10 hr rate (1.37 $\mu\text{mole N} \times \text{gdw RR}^{-1} \times \text{hr}^{-1}$) ($p \leq 0.001$) (Table 8). Uptake rates ranged 0.84-3.30 $\mu\text{mole N} \times \text{gdw RR}^{-1} \times \text{hr}^{-1}$ and averaged 1.76 $\mu\text{mole N} \times \text{gdw RR}^{-1} \times \text{hr}^{-1}$ (Table 8). Uptake of $^{15}\text{NH}_4^+$ was not found to be concentration dependent for the 4 hr isotope exposure time ($p \geq 0.1$) but was concentration dependent during the 10 hr as T1 was different than T3 ($p \leq 0.05$) (Table 7 and Figure 9f). The 4 hr $V_{\max} = 3.32$ and $K_s = 44.8$ while the 10 hr $V_{\max} = 3.02$ and $K_s = 99.5$ (Table 9).

In all three experiments (GHEX 2, 3, 4) uptake rate was a function of available NH_4^+ for one of the two incubation intervals tested (4 hr= GHEX 2 and 3; 10 hr= GHEX 4). In all three experiments the 4 hr mean rate was greater than the 10 hr mean rate (Table 8). Uptake rate was a function

of both incubation time and external concentration and the null hypotheses presented in Section C, objective #3 were rejected.

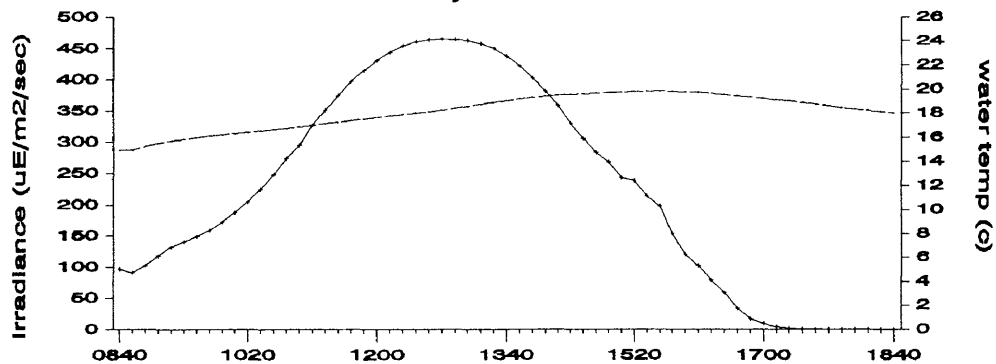
Table 6. Experimental conditions for Greenhouse Root-Rhizome $^{15}\text{NH}_4^+$ uptake Experiments (GHEX). Eastern standard time, irradiance (PAR= $\mu\text{Einsteins x m}^{-2} \text{ x sec}^{-1}$), and water temperature in $^{\circ}\text{C}$. Light sensor and thermistor placed in tank 2. (see Figure 2).

LABEL	DATE	TIME	PAR		TEMP	
			Range	Avg	Range	Avg
GHEX2	2Nov90	0840-1840	96.5-464.5	224.0	15.0-19.8	18.1
GHEX3	20Feb91	0820-1820	50.3-201.0	77.4	10.1-11.2	10.5
GHEX4	25Apr91	0800-1800	81.0-634.3	374.1	13.4-18.9	17.3

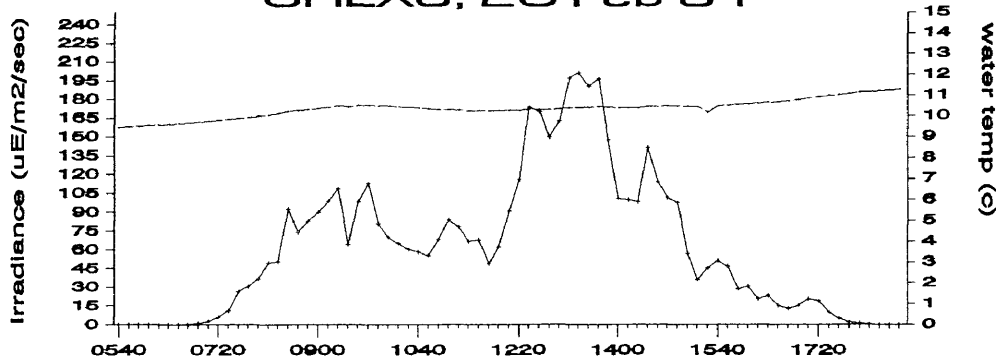
Figure 8. Plot of experimental conditions during GHEX
2, 3, and 4. Times are reported in EST.

—— = PAR - - - - = TEMP

GHEX2, 2 Nov 90



GHEX3, 20 Feb 91



GHEX4, 25 Apr 91

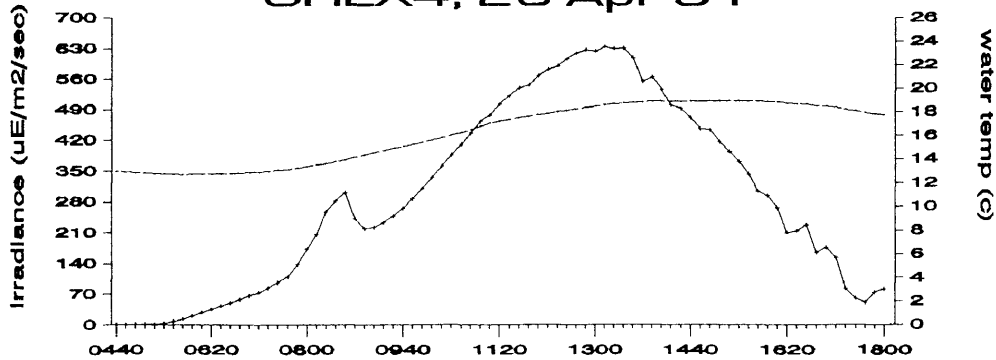


Table 7. Initial, 4 hour, and 10 hour plant ^{15}N atom excess; plant accumulation of ^{15}N ($\mu\text{mole N} \times \text{gdwRR}^{-1}$); and plant $^{15}\text{NH}_4^+$ uptake rates ($\mu\text{mole N} \times \text{gdwRR}^{-1} \times \text{hr}^{-1}$) derived from greenhouse experiments ($n=5$, mean \pm sd). Controls injected with $^{14}\text{NH}_4^+$ while Treatments 1, 2, 3 correspond to $^{15}\text{NH}_4^+$ concentrations representative of average sediment ammonium range from field site (μM). Plant $^{15}\text{N}\%$ reported are corrected JASCO ^{15}N machine values. Uptake rates were calculated using the following RR %N: GHEX2 and GHEX3=1.4% N, GHEX4=0.94% N. (*) $n=2$.

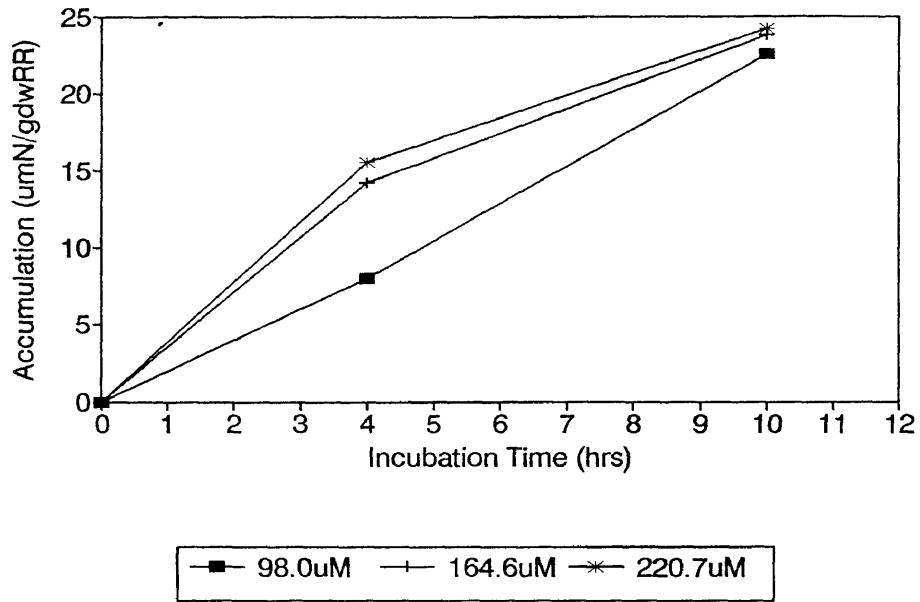
	PLANT ^{15}N %		ACCUMULATION		UPTAKE RATE		
	(0hrs) mean \pm sd	(4hrs) mean \pm sd	(10hrs) mean \pm sd	(4hrs) mean \pm sd	(10hrs) mean \pm sd	(4hrs) mean \pm sd	(10hrs) mean \pm sd
GHEX2 2 Nov 90							
C(172.2)	0.226 \pm 0.11	0.280 \pm 0.09	0.215 \pm 0.07	n/a	n/a	n/a	n/a
T1(98.0)	0.314 \pm 0.13	0.723 \pm 0.25	2.028 \pm 0.41	8.03 \pm 2.76	22.53 \pm 4.54	2.01 \pm 0.69	2.25 \pm 0.45
T2(164.6)	0.269 \pm 0.11	1.283 \pm 0.34	2.140 \pm 0.66	14.25 \pm 3.80	24.37 \pm 7.39	3.56 \pm 0.95	2.38 \pm 0.74
T3(220.7)	0.393 \pm 0.13	1.402 \pm 0.24	2.179 \pm 0.25	15.58 \pm 2.62	24.21 \pm 2.77	3.90 \pm 0.66	2.42 \pm 0.28
GHEX3 20 Feb 91							
*C(43.9)	0.260 \pm 0.09	0.310 \pm 0.04	0.297 \pm 0.08	n/a	n/a	n/a	n/a
T1(23.2)	0.568 \pm 0.15	0.599 \pm 0.14	1.259 \pm 0.30	6.65 \pm 1.50	13.99 \pm 3.37	1.66 \pm 0.38	1.40 \pm 0.34
T2(45.5)	0.516 \pm 0.12	0.766 \pm 0.18	1.291 \pm 0.40	8.51 \pm 1.97	14.34 \pm 4.39	2.13 \pm 0.49	1.43 \pm 0.44
T3(83.2)	0.512 \pm 0.13	0.933 \pm 0.24	1.849 \pm 0.55	10.36 \pm 2.70	20.54 \pm 6.07	2.59 \pm 0.68	2.05 \pm 0.61
GHEX4 25 Apr 91							
*C(93.7)	0.262 \pm 0.04	0.203 \pm 0.13	0.196 \pm 0.11	n/a	n/a	n/a	n/a
T1(58.8)	0.286 \pm 0.12	0.736 \pm 0.19	1.109 \pm 0.13	6.92 \pm 1.80	10.43 \pm 1.26	1.73 \pm 0.45	1.04 \pm 0.13
T2(80.5)	0.199 \pm 0.07	1.017 \pm 0.21	1.555 \pm 0.25	9.57 \pm 1.98	14.63 \pm 2.31	2.39 \pm 0.49	1.46 \pm 0.23
T3(119.6)	0.223 \pm 0.10	0.982 \pm 0.31	1.606 \pm 0.50	9.23 \pm 2.94	16.06 \pm 5.00	2.31 \pm 0.74	1.61 \pm 0.50

Table 8. Minimum, maximum, and mean uptake rates recorded over all plant incubation chambers ($N_{\text{total}} = 30$) and over all 3 treatments for each incubation time ($n_{4\text{hr}} = 15$; $n_{10\text{hr}} = 15$) during GHEX 2, 3, and 4. Rates are reported in $\mu\text{mole N} \times \text{gdw RR}^{-1} \times \text{hr}^{-1}$.

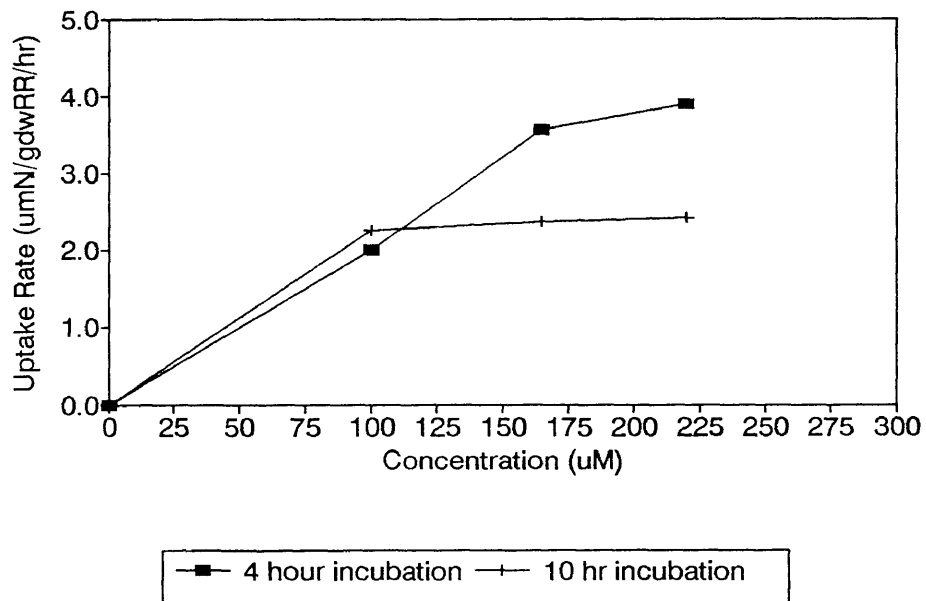
EXPERIMENT		MIN	MAX	MEAN
GHEX 2 Nov 90	N_{tot}	0.780	5.024	2.753
	$n_{4\text{hr}}$	0.780	5.024	3.155
	$n_{10\text{hr}}$	1.545	3.559	2.351
GHEX 3 Feb 91	N_{tot}	0.726	3.331	1.878
	$n_{4\text{hr}}$	1.284	3.331	2.127
	$n_{10\text{hr}}$	0.726	2.762	1.629
GHEX 4 Apr 91	N_{tot}	0.843	3.302	1.757
	$n_{4\text{hr}}$	1.382	3.302	2.143
	$n_{10\text{hr}}$	0.843	2.216	1.371

Figure 9. Root/Rhizome ^{15}N accumulation and uptake
(a-f) recorded during GHEX 2, 3, and 4. Data points
represent mean values for each combination of
incubation time and NH_4^+ concentration (n=5).

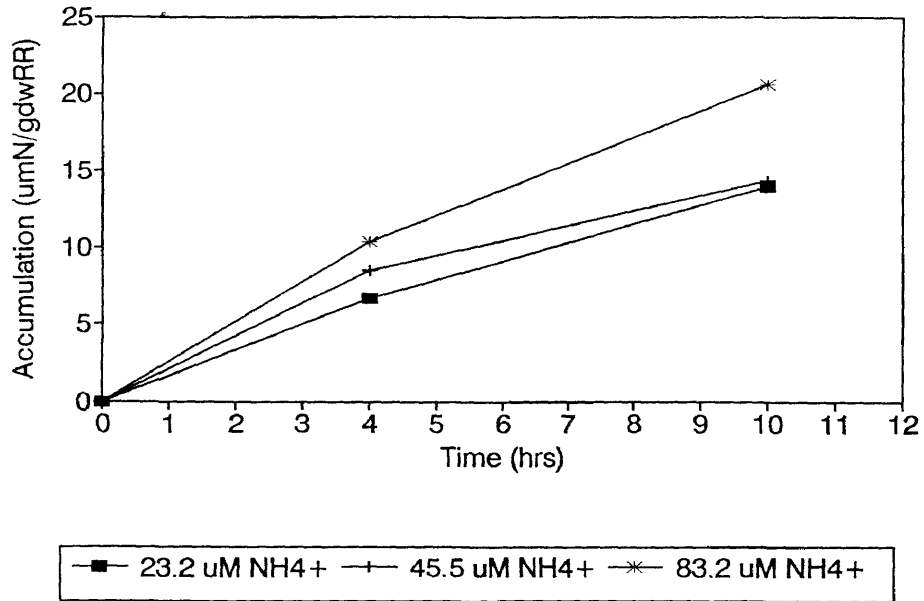
(9a) ROOT/RHIZOME N ACCUMULATION
GHEX-2, NOV,90 Accum vs Time



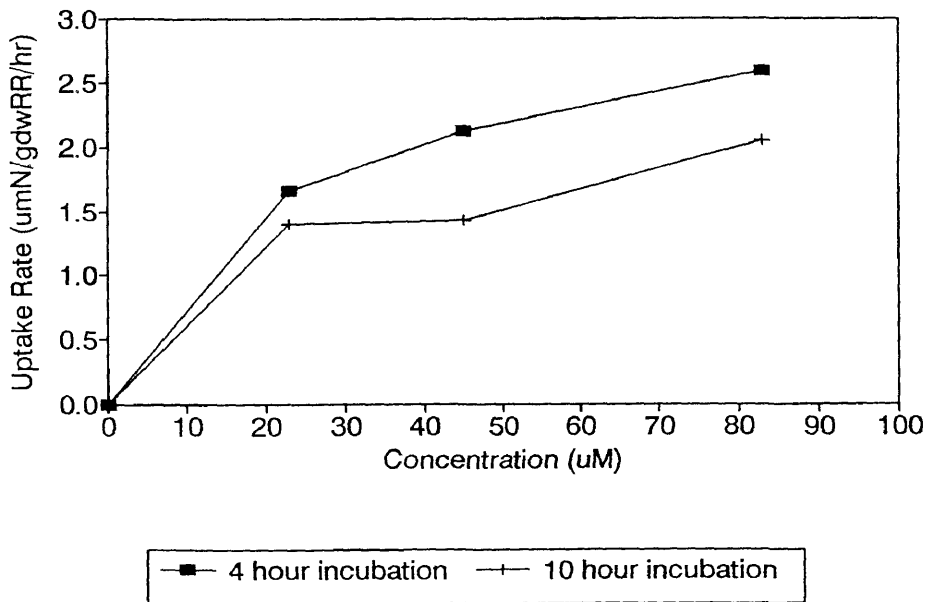
(9b) ROOT/RHIZOME NH₄⁺ UPTAKE
GHEX-2, NOV,90 Rate vs NH₄⁺



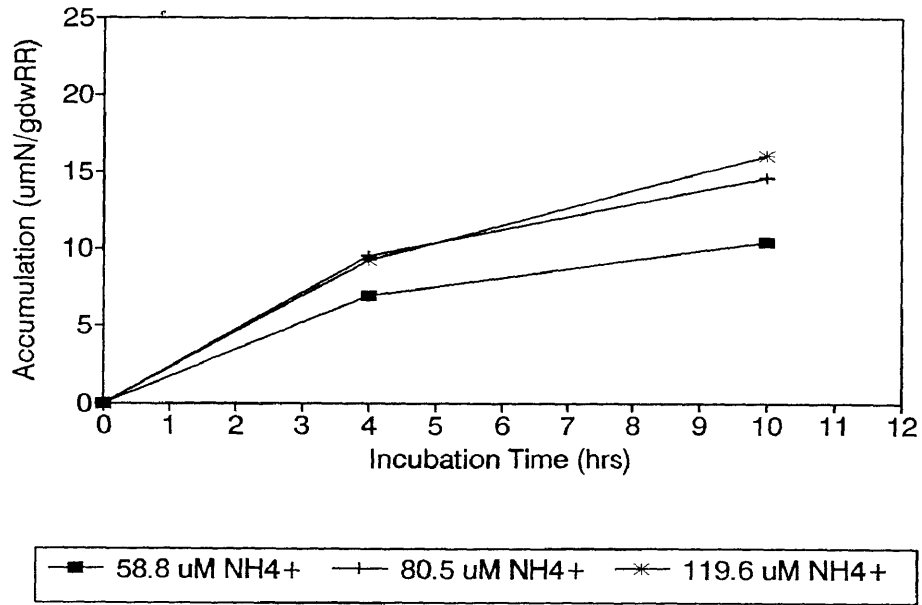
(9c) ROOT/RHIZOME N ACCUMULATION
GHEX-3, FEB,91 Accum vs Time



(9d) ROOT/RHIZOME NH4+ UPTAKE
GHEX-3, FEB,91 Rate vs NH4+



(9e) ROOT/RHIZOME N ACCUMULATION
GHEX-4, APR,91 Accum vs Time



(9f) ROOT/RHIZOME NH4+ UPTAKE
GHEX-4, APR,91 Rate vs NH4+

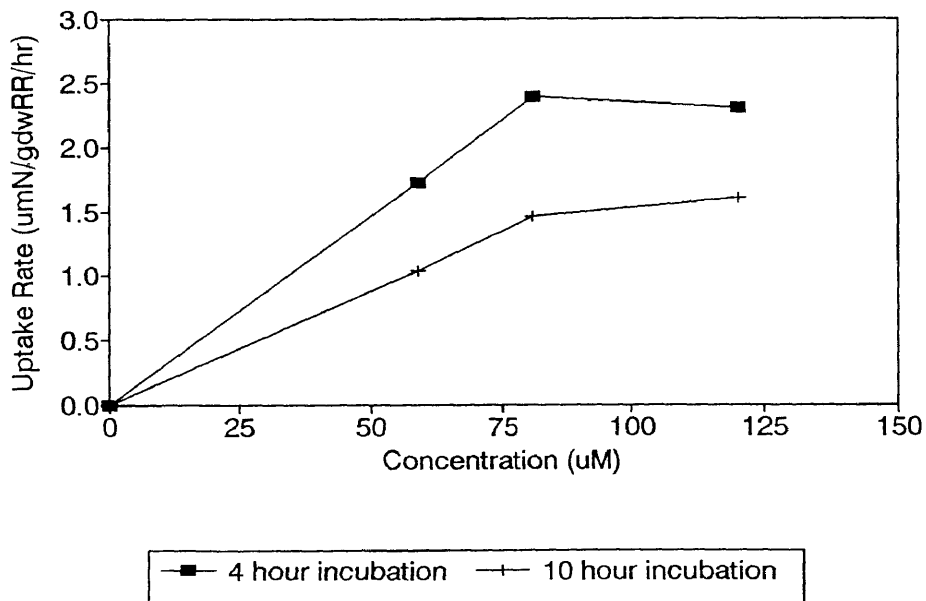


Table 9. Michaelis-Menten kinetic parameters for greenhouse experiments (GHEX). V_{\max} is the estimated maximum rate of $^{15}\text{NH}_4^+$ uptake ($\mu\text{moles N} \times \text{gdw RR}^{-1} \times \text{hr}^{-1}$) while K_s is the substrate concentration (μM) where $V=(0.5)(V_{\max})$. Experimental physical parameters and treatment levels are shown in Tables 6 and 7, respectively.

LABEL		V_{\max}	K_s
GHEX2	4hr.	12.5	461
Nov 90	10hr.	2.58	14.1
GHEX3	4hr.	3.29	23.4
Feb 91	10hr.	2.43	21.0
GHEX4	4hr.	3.32	44.8
Apr 91	10hr.	3.02	99.5

B. DISCUSSION

The monthly relationships between plant biomass, sediment organic matter, sediment NH_4^+ and NO_x^- concentrations, and plant nitrogen content suggest bi-directional influence between eelgrass and its sediment environment. As the plant progresses through its growth cycle, senescent shoots, root-rhizomes and trapped allochthonous organic materials are deposited into the meadow sediment. NH_4^+ is remineralized from this organic matrix and retained. Sediment organic content is a function of plant biomass but in turn affects in situ NH_4^+ concentrations. Shoot C:N closely tracks plant biomass and is statistically related to sediment NH_4^+ ($r^2=0.313$, $p \leq 0.002$; Figure 7e). Although no parallel statistical relationship could be drawn between biomass and NH_4^+ ($r^2=0.01$, $p \geq 0.07$), biomass increase and subsequent decline indirectly affect sediment NH_4^+ concentrations down to 10 cm in depth. This lack of direct coupling between plant biomass and sediment NH_4^+ production suggests that there is perhaps a time lag between plant growth and increased sediment ammonification. Sediment NH_4^+ is the primary environmental nitrogen source for eelgrass and its availability is a possible limiting factor to net productivity as the plants must utilize internal nitrogen reserves.

Sediment NO_x^- is vertically and monthly variable. Although this study did not analyze adjacent unvegetated

areas, previous sediment coring in unvegetated patches of the eelgrass meadow near the sampling plot of this study have similar monthly extractable NO_x^- concentrations (Wetzel, Morris, and Berry, unpubl. data). The autumn-early winter NO_x^- maxima is believed to result from sediment nitrification due to rhizosphere oxygenation through shoot photosynthesis and basipetal oxygen transport and decreased sediment oxygen demand from a decline in labile organic matter during this time of year. This finding is consistent with eelgrass sediment nitrification data from the beds near this study site (Wetzel, Morris, Berry unpubl. data) and of the eastern side of the lower Chesapeake Bay (Caffrey and Kemp 1990).

Plant biomass values reported here are higher than previously reported for the lower Chesapeake Bay. They are likely overestimates since the plants were not separated into living and dead components and the values represent all the plant matter contained in the core. Because the analytical samples used in C:N ratio determinations were homogenized groups of living and dead shoot or root-rhizome tissue, the C:N determinations could be biased and may be over or underestimates. Differently aged leaves, roots, and rhizome segments can vary in their carbon and particularly nitrogen content (Pirc and Wollenweber 1988; Borum et al. 1989). Despite these characteristics of the samples, the C:N data are reasonable except for the June CNRR because of

the overall agreement between the %carbon, %nitrogen, and C:N molar ratios with established literature values for eelgrass (Short 1987; Borum et al. 1989; Duarte 1990).

The C:N root-rhizome data supports the internal translocation-reclamation scenario of Iizumi and Hattori (1982) and Borum et al. (1989). The high summer and winter RR nitrogen content decreases dramatically during times of increased plant growth as internally stored nitrogenous organic compounds are translocated to the above ground tissue. The amount of internal translocation and reclamation of nitrogen by eelgrass is inversely related to nitrogen availability in the environment (Borum et al. 1989).

The root-rhizome NH_4^+ uptake experimental design was similar to others that have been employed. The number of plants per treatment and experimental NH_4^+ concentrations were like those of Short and McRoy (1984) and Williams and Fisher (1985). The 4 and 10 hr incubation times were adapted from review of these two publications and Iizumi and Hattori (1982). Short and McRoy used smaller time intervals (ca 15 minutes) up to 4 hrs to derive their leaf uptake rates while measuring leaf ^{15}N accumulation up to 25 hrs (1984). The most rapid leaf absorption occurred over the initial ≤ 15 minutes of isotope exposure while accumulation was undetectable after 14 hrs (Short and McRoy 1984). Root-rhizome uptake rates were determined after 4

hrs of incubation with ^{15}N . Williams and Fisher used 5 incubation times (0.5-6.0 hrs) to measure ^{15}N uptake by the tropical green alga *Caulerpa cupressiodes* (1985) while Iizumi and Hattori incubated eelgrass up to 24 hrs in their investigation of uptake and translocation of ^{15}N (1982). As a compromise to both time and money, the 4 and 10 hr incubation times were chosen for this study. Based upon these results the absolute leaf and RR kinetic parameters, V_{\max} and K_s , are most accurately determined from incubations up to 4 hours with 15-30 minute sampling intervals.

There are some potential problems in determining uptake rates with the methods of this study. Apparent plant ^{15}N enrichment could have either been from adsorption to the root-rhizome surface or from actual uptake and accumulation since no specific experiments were done to differentiate between these effects (Williams and Fisher 1985). It is also unknown if the ^{15}N measured is part of an internal NH_4^+ pool or is part of plant organic compounds. In the event that the t_0 ^{15}N pool was diluted due to the extraneous presence of $^{14}\text{NH}_4^+$ (from the plastic plant flasks, severed root-rhizomes, rhizosphere bacteria, etc.), the uptake rates reported could actually be underestimates (Williams and Fisher 1985). The severing of root-rhizomes during the plant selection process could have had an effect upon measured uptake rates. This could be avoided by growing the plants from seed when doing controlled experiments. Given

the higher RR nitrogen contents of the fall and winter vs the spring plants, the fall and winter uptake rates could be underestimates due to dilution of the isotope in the tissue. The 10 hr uptake rates could be underestimates as well because it is believed that 10 hrs is sufficient time for some internal translocation to occur. For this reason only the 4 hr kinetic parameters are compared between the three experiments. The uptake rates derived from these experiments are similar to those of Short and McRoy (1984) but are generally less than those of Iizumi and Hattori (1982) for eelgrass and Williams and Fisher (1985) for *Caulerpa cupressiodes*.

Short and McRoy (1984) state that if the relationship between uptake rate and concentration follows Michaelis-Menten kinetics, then absorption into the internal cell NH_4^+ pool limits uptake. Iizumi and Hattori (1982) state that above 100 μM NH_4^+ uptake is limited by diffusion across the root surface. This would imply a linear relationship between uptake rate and external concentration. The data here suggest a Michaelis-Menten relationship and therefore uptake rate should be limited by ion absorption into the internal cell pool. These data do not imply anything about the actual uptake mechanism. The mode of uptake (active vs passive), the uptake potential of roots vs rhizomes, and the actual sites of nitrogen absorption are all unknown entities.

GHEX 2 showed a greater 4 hr mean uptake rate over all treatments than either GHEX 3 or 4 (3.16 $\mu\text{mole} \times \text{gdw RR}^{-1} \times \text{hr}^{-1}$ vs 2.13 or 2.14). Since uptake was shown to be concentration dependent, the greater rates of this fall experiment could be due to the larger NH_4^+ concentrations tested. But the experimental NH_4^+ concentrations tested were representative of field concentrations. Therefore it can be concluded that fall uptake rates are greater than those of the winter or spring. Winter uptake rates were similar to those of the spring despite lower NH_4^+ concentrations. Estimation of uptake rates for field populations derived from the laboratory kinetic parameters and in situ NH_4^+ concentrations suggest that sediment NH_4^+ is in excess of plant uptake potential in both winter and spring. It appears that *Zostera marina* L. is capable of "luxury uptake" during times of high sediment NH_4^+ and decreased plant N demand in the fall and winter. This nitrogen is stored internally in root-rhizome tissue for acropetal translocation in the spring when plant N demand increases due to increased production and biomass (Figure 5).

An eelgrass seasonal nitrogen budget was derived to analyze plant nitrogen demand vs sediment supply (Table 10). The budget was created on a shoot $\times \text{day}^{-1}$ basis using data from this study (leaf weight $\times \text{shoot}^{-1}$; RR wt $\times \text{shoot}^{-1}$; nitrogen contents of leaves and RR; plant NH_4^+ uptake rates;

sediment NH_4^+ concentrations), Orth and Moore (1986) (shoot densities), Caffrey and Kemp (1990) (rates of ammonification, nitrification, nitrate reduction), and Moore et al. (in press) for net shoot and RR growth rates. The budget is based on net accumulation of biomass (growth-loss). During the winter net growth=net loss therefore no nitrogen budget was derived. There were many assumptions made in the creation of this eelgrass nitrogen budget. Net growth, net loss, shoot density, leaf weight x shoot⁻¹, RR weight x shoot⁻¹, nitrogen content of leaves and RR, RR nitrogen uptake rates, sediment ammonification, nitrification, and dissimilatory nitrate reduction were all integrated values and were assumed to be constant over each season. Since the summer GHEX 1 data were unusable, the uptake rate for this season was estimated to be less than the other seasons because of the inhibition of eelgrass physiology during the summer. Missing from the calculations are the roles of leaf NH_4^+ and NO_x^- uptake, RR NO_x^- uptake, internal N translocation, diffusion of NH_4^+ to the water column, denitrification, and nitrogen fixation. The sediment NH_4^+ concentrations reported in Table 10 are the result of the following conversions;

$$\text{Sediment } \text{NH}_4^+ = (\text{NH}_4^+) + (\text{ammonification}) + (\text{dissimilatory } \text{NO}_3^- \text{ reduction}) - (\text{nitrification}) - (\text{root/rhizome uptake})$$

Where: Sediment $\text{NH}_4^+ = \text{mmol} \times \text{m}^{-2}$
 ammonification, NO_3^- reduction,
 nitrification, and uptake = $\text{mmol} \times \text{m}^{-2} \times \text{day}^{-1}$

Table 10. Seasonal eelgrass nitrogen budget. This budget is based on net production (growth - loss; $\text{gdw} \times \text{gdw}^{-1} \times \text{day}^{-1}$). Shoot density = shoots $\times \text{m}^{-2}$; Leaf and root-rhizome weights = $\text{gdw} \times \text{shoot}^{-1}$; nitrogen content = $\text{gN} \times \text{gdw plant}^{-1}$; uptake rate, ammonification, nitrification, dissimilatory reduction = $\text{mmole N} \times \text{m}^{-2} \times \text{day}^{-1}$. Nitrogen demand (Ndem) of the leaf, RR, and plant = $\text{mmolN} \times \text{m}^{-2}$. NH_4^+ = $\text{mmolN} \times \text{m}^{-2}$ and sediment NH_4^+ (Sed NH_4^+) results from (initial concentration + ammonification + dissimilatory NO_x^- - nitrification - plant uptake).

Seas	Net Prod g/g/d	Shoot Dens. sh/m ²	Leaf Wt. gdw/l	RR Wt. gdw/RR	Nit cont. Leaf % dw	Nit cont. RR % dw	NH_4^+ mmol N/m ²	NH_4^+ Prod mmol N/m ² /d	NO_3^- Prod mmol N/m ² /d	Diss NO_3^- mmol N/m ² /d
Sum	0.003	1217	0.101	0.055	0.019	0.015	10.0	28.7	18.6	24.0
Fall	0.008	1233	0.070	0.061	0.024	0.012	8.4	19.0	35.0	55.2
Spr	0.035	1800	0.128	0.063	0.024	0.012	6.0	24.2	10.1	100.0
	Ndem Leaf mmol N/m ²	Ndem RR mmol N/m ²			Ndem Plant mmol N/m ²	Uptake Rate mmol N/m ² /d	Sed NH_4^+ mmol N/m ²			
Sum	0.546	0.234			0.780	19.27	24.81			
Fall	1.216	0.552			1.769	43.33	4.23			
Spr	13.54	3.260			16.80	43.55	76.59			

Note: Sediment NH_4^+ is estimated on a daily basis and is assumed to be constant over entire season.

It appears that sediment NH_4^+ is in excess of whole plant net growth demand for the summer, fall, and spring seasons (Table 10). This assertion is supported by Kenworthy et al. (1982) and Dennison et al. (1987) where sediment NH_4^+ production and concentrations were greater than plant nitrogen requirement. The fall and the spring are the most active times of eelgrass sediment nitrogen conversions as nitrification and increased rates of uptake decrease sediment NH_4^+ in the autumn and increased dissimilatory NO_x^- and plant nitrogen demand influence sediment NH_4^+ in the spring. Based on this budget eelgrass seasonal nitrogen demand does indeed vary considerably. The reader is cautioned not to accept the results of this exercise too literally. The wide variety of the analytical methods, the problems inherent with manipulative experiments, the blanket assumptions employed, and the overall small size of the data set make this budget less than ideal.

From their model of sediment nitrogen supply and eelgrass production, Zimmerman et al. (1987) concluded that eelgrass is not nitrogen limited. These conclusions are spurious due in part to the unrealistic V_{max} and K_s values they employed in their model. These parameters were taken from Thursby and Harlin (1982) who used large volume jars,

NH_4^+ disappearance rates, and long term experiments to derive them. Large volume jars and long term experiments may allow for NH_4^+ production by extraneous bacteria and the NH_4^+ can accumulate in many other places besides the plant tissue. The plant flask total NH_4^+ concentrations at time₀ (0 hrs), time₁ (4 hrs), and time₂ (10 hrs) from all three experiments of this study reflect only slight changes in absolute concentration although significant uptake was measured by isotope incorporation.

Nitrogen supply vs plant nitrogen demand is only one component of the apparent nitrogen limited eelgrass production puzzle. Because sediment NH_4^+ was in excess of plant requirement for growth, Dennison et al. (1987) and Kenworthy et al. (1982) concluded that eelgrass was not nitrogen limited. Environmental regulatory factors such as competition for resources (e.g. light and nutrients), temperature, diffusion barriers, sediment anoxia, or toxic pollutants can affect the ability of the plants to utilize available external NH_4^+ concentrations.

As in this study, Caffrey and Kemp (1990) did not sample the actual sediment rhizosphere to determine the rates of nitrogen cycling. This methodology could introduce bias because the daily oxygenation of the rhizosphere produced by plant photosynthesis creates the microhabitats of nitrogen transformation. The potential impact of nitrification and denitrification is not

sufficiently quantified using these methods. Caffrey and Kemp (1990) and Dennison et al. (1987) point to the possible role of rhizosphere hypoxia-anoxia and sulfides in regulating sediment nutrient cycling. This is probably important, particularly in the fall as sediment sulfides seem to increase dramatically and the plant NH_4^+ uptake potential is not saturated. Pregnall et al. (1984) demonstrated the necessity for rhizosphere oxygenation in eelgrass NH_4^+ uptake by the internal presence of glutamate and glutamine in the root tissue during oxic but not during anoxic conditions. Energy produced aerobically through the Krebs cycle is necessary in attaching NH_4^+ to glutamate to form glutamine.

More work is needed several areas before the question of possible eelgrass nitrogen limitation can be answered.

These areas include:

- (1) Thorough measurement of eelgrass in situ shoot and root-rhizome productivity on a seasonal basis.
- (2) Analysis of sediment sulfide and rhizosphere hypoxia/anoxia and their effects upon eelgrass nitrogen uptake kinetics.
- (3) Proper experiments to pinpoint the role of internal nitrogen translocation and reclamation in eelgrass nitrogen nutrition.
- (4) Analysis of the nitrogen transformations, including denitrification, that occur in the actual sediment rhizosphere in eelgrass beds.
- (5) Investigation into the role of phosphorous nutrition and how it relates to the nitrogen cycle.

Cycles of plant growth and decomposition in coastal macrophytic communities have a profound impact upon the chemical composition of the sediments. Plant photosynthesis, uptake processes, and biomass decline influence the sediment carbon, nitrogen, and phosphorus biogeochemical cycles. However, sediment microbial usage of deposited organic matter creates zones of diminished or inaccessible nutrient concentrations. This can inhibit plant production by decreasing essential inorganic nutrient availability. Plant-sediment interaction is an excellent example of the dynamic bi-directional relationship between an organism and its environment.

IV. CONCLUSIONS

- (1) Eelgrass meadow sediment inorganic nitrogen (NH_4^+ and NO_x^-) varies monthly and seasonally.
- (2) Eelgrass nitrogen requirement for net biomass accumulation varies seasonally.
- (3) Eelgrass root-rhizome NH_4^+ uptake rates are concentration dependent over a seasonal range of sediment NH_4^+ .
- (4) Incubation time is an important consideration in the design of eelgrass NH_4^+ uptake experiments when determining kinetic parameters.
- (5) The roles of internal nitrogen translocation, the specific rhizosphere nitrogen transformations, phosphorous nutrition and its relationship to the nitrogen cycle, and the inhibitory influence of sediment sulfide all require further investigation before the question of eelgrass nitrogen limited production can be answered.

APPENDIX

In June 1990, September 1990, January 1991, and April 1991 a total of six sediment cores were extracted from the field site. This was done in order to compare the variability in the determination of sediment inorganic nitrogen using three vs six sediment cores. For each of the months specified, the cores were numbered 1-6 and three were randomly selected. A mean NH_4^+ concentration was calculated for these three cores and was compared to the six core average using a t-test in each of the months. The coefficient of variation (cv) for the three core average was also compared to that of the six core average.

Table A. Comparison of mean NH_4^+ concentrations (μM) between three vs six sediment cores (0-10 cm). The t-probability (t-prob) is the probability resulting from the t-statistic calculated using the pooled variances.

Month	N	Mean±sd	cv	t-prob
June	3	349.28±78.41	0.224	0.435
	6	399.39±88.22	0.221	
Sept	3	188.66±44.41	0.235	0.447
	6	241.26±105.64	0.438	
Jan	3	174.81±82.01	0.469	0.908
	6	169.15±59.43	0.351	
April	3	109.39±45.66	0.417	0.638
	6	123.16±36.91	0.300	

From this table it is apparent that for this timing of sampling and these analytical methods there is no statistical advantage in selecting six cores rather than three. The greatest difference between the two means occurred in June where total sediment NH_4^+ concentrations were greatest. I believe if a total of 10 or 12 cores were taken and compared to the 3 core average a significant difference would occur. Of course there are several factors that influence these results. The natural variability of NH_4^+ distribution is a possible factor. The cores were not randomly selected in the field in an effort to standardize the coring technique. This is potentially a large influence upon the results. Since the three cores that were used in the comparison were randomly selected from the group of six, it is possible that another combination of three could produce quite different results.

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